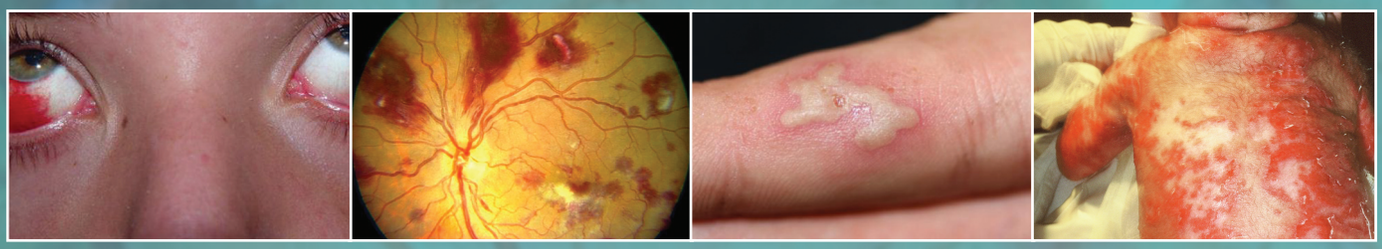
Mucocutaneous Manifestations of Viral Diseases

Second Edition



Edited by
Stephen K Tyring
Angela Yen Moore
and
Omar Lupi

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Preface to the second edition

The skin is the window of the body. Many viral diseases express themselves through changes in skin appearance, skin lesions, edema, etc. Often the skin becomes a warning of internal manifestations signaling the physician to look beyond the window for other impacts. The contributors to the second edition of ***Mucocutaneous Manifestations of Viral Diseases*** interact on a regular basis with other dermatologists and virologists, or combinations thereof. The book is an outgrowth of those daily consultations and discussions with other colleagues in various fields of medicine. As family practitioners are increasingly being called upon to provide their diagnosis and treatment of a wider variety of illnesses (those previously referred to a specialist), a quick reference is needed. This book not only helps distinguish the cutaneous manifestations of one virus from another, but also helps differentiate viral diseases from other infectious and noninfectious diseases. It is intended for internists, dermatologists, pediatricians, and family practitioners worldwide.

The goal of this book is to enhance the expertise of physicians in the diagnosis, treatment and pathogenesis of viral diseases that express their presence in the skin and its affiliated mucous membranes. No other text currently addresses the issues of the skin manifestations of viral diseases. Photographs in other texts to aid diagnoses were previously black and white and of limited use to the physician. Many color atlases only encompass one or a few viral diseases, leaving the practioner with a desire for more detail and/or a better explanation of possible mimics of the diseases.

The contributing authors and editors have provided a text that serves as a central resource for each of the viral diseases described. It should be of interest to physicians worldwide as we have included many diseases previously known only in third world, developing countries. Given the global aspects of international transportation, social exchange and political boundaries, it is not only feasible that one or more of these rarer viral diseases could present itself at any physician's office in the world, many examples of this occurrence have been documented since the first edition of this book was published. Animal vectors and reservoirs are often immigrants on baggage or agricultural products. Each chapter includes, as appropriate, a timeline of infection and progress of the disease, numerous quality color illustrations of characteristic epidermal and cellular manifestations, a means to reference the differential diagnosis of viral diseases from other infectious or noninfectious diseases, a brief taxonomy and history of the disease, incidence among gender and age groups by geographical region, pathogenesis, clinical manifestations, dermatopathology, laboratory findings, differential diagnoses, and treatment/prophylaxis. To the extent possible, we have used tabular information for quick reference by the physician.

The second edition of ***Mucocutaneous Manifestations of Viral Diseases*** is unique in that it covers the field of viral diseases having mucocutaneous manifestations and offers the quality color photographs associated with an atlas. The book also serves as a bibliography for physicians wishing to broaden their knowledge of the primary literature. We envision the physician using the color photographs in considering the possible diagnoses. The differential diagnosis section helps the physicians narrow the search for the virus causing the epidermal insult. The text would then provide suggestions as to which laboratory tests might be useful to confirm the diagnosis. Finally, it outlines the appropriate treatment, including specific types of antiviral drugs and vaccines.

In summary, the editors hope that the second edition of ***Mucocutaneous Manifestations of Viral Diseases*** will fill a void in the medical literature and provide a valuable resource to a variety of practicing physicians worldwide.

Stephen K Tyring
Angela Yen Moore
Omar Lupi

1 Cutaneous Virology

Stephen K Tyring

Introduction

Viral diseases may produce mucocutaneous manifestations either as the result of viral replication in the epidermis or as a secondary effect of viral replication elsewhere in the body. Most primary epidermal viral replications result from three groups of viruses: human papillomaviruses (HPV), herpesviruses, and poxviruses. Secondary skin lesions are produced by such virus families as retroviruses, paramyxoviruses, togaviruses, parvoviruses, and picornaviruses. Rhabdoviruses, rotavirus, etc., rarely induce skin lesions and are beyond the scope of this book. The mucocutaneous manifestations of subviral agents, such as viroids and prions, are only beginning to be understood.

A number of cutaneous diseases appear to be viral exanthemas, but no virus has been proven to be the etiologic agent in some of these diseases. For example, pityriasis rosea (PR) is an acute, self-limiting, cutaneous eruption with a distinctive course. The initial lesion, the herald patch, is followed after 1–2 weeks by a generalized secondary rash, which typically lasts about 6 weeks (Fig. 1.1). Like most viral infections, PR shows seasonal variability, with an increased incidence in the autumn and winter and a decreased incidence in the summer. A preceding upper respiratory infection is often noted with PR, as are clusters of cases in time and space. Recently, PR was hypothesized to be due to infection with human herpesvirus 7, and most controlled studies have supported this hypothesis.

Likewise, asymmetric periflexural exanthem of childhood (APEC) or unilateral laterothoracic exanthem (ULE) is suspected to be of viral etiology. It presents in children, six months to five years of age, in the winter and spring. The rash is unilateral on the trunk, often in the axillae or large flexures of the limbs (Figs. 1.2 and 1.3). It spreads centrifugally and to the contralateral side over 2–4 weeks and resolves in 6 weeks. Initially, red, 3-mm papules appear, followed by a scarlatiniform or eczematous rash. There are no constitutional symptoms, but an enlarged lymph node is usually observed at the primary site. Viruses that are suspected, but not proven to be of etiologic significance, include parvovirus B-19, parainfluenza 2 or 3, and adenoviruses.

On the other hand, several new viral diseases, or viral diseases in new geographic areas have been described recently. These diseases include infections due to Lujo virus (an arenavirus), bocavirus (a parvovirus), bannavirus (a reovirus), TTV (a circovirus), Nipah virus and metapneumovirus (paramyxoviruses), and zika virus (a flavivirus). When cutaneous manifestations of such viruses are reported, the description in the general medical literature is rarely more specific than "rash" or "skin rash." More specific descriptions in the dermatology literature might aid in the more rapid diagnosis of these diseases.

Clinical Manifestations

Viral infections can result in a wide spectrum of skin lesions. HPV infection frequently results in verrucous papules, but the range of presentations includes erythematous macules in epidermodysplasia verruciformis, smooth papules in bowenoid papulosis, and fungating Buschke-Lowenstein tumors. The primary lesions in herpes simplex virus (HSV), varicella-zoster virus (VZV), and many coxsackievirus infections are vesicles. Erythema and papules often precede the vesicles, which are followed by pustules, crusts, or shallow ulcers. Cytomegalovirus (CMV) infections of the skin and mucous membranes, as well as HSV, VZV, or coxsackievirus infections of mucous membranes, can present as ulcers without other stages. Measles and rubella can be associated with both macules and papules. Epstein-Barr virus (EBV), human herpesvirus type 6 (HHV-6), and parvovirus B19 infections may result in macules that coalesce into larger erythematous patches. A spectrum of nonspecific skin lesions, such as erythema multiforme, urticaria, and petechiae, may be viral or nonviral in etiology. Mucocutaneous manifestations of viral diseases can range from very specific (e.g., dermatomal vesicles of herpes zoster) to very general (e.g., urticaria); thus, the differential diagnosis must take the total clinical presentation of the patient into consideration (Table 1.1). Some skin changes may be highly suggestive of a specific viral disease, such as the verrucous papules seen with papillomavirus infection or smooth umbilicated papules resulting from poxvirus infection. Often, further diagnostic tests may not be needed for these conditions. A differential diagnosis, including both viral and nonviral etiologies, may be suggested by vesicles induced by HSV-1 or -2 or VZV or they may be diagnostic. The diagnosis may not be obvious when any of these three viruses produce mucous membrane lesions and further diagnostic procedures may be required. Less frequent skin manifestations may be produced by other herpes viruses, such as EBV, CMV, and HHV-6. These infections are most accurately diagnosed only when the systemic manifestations of the viral infection are simultaneously considered. The cutaneous manifestations would indicate the need to evaluate systemic signs and symptoms and to institute appropriate diagnostic tests in other diseases where viral replication is not in the epidermis.

Pathophysiology

Three different routes are used by viruses to infect the skin: direct inoculation, local spread from an internal focus, or systemic infection. Papillomaviruses, most poxviruses, and primary HSV infect the skin by direct inoculation. The skin in primary VZV is infected from systemic infection, while recurrent VZV (shingles) or recurrent HSV reaches the skin from an internal focus.

Skin lesions may be the direct effect of virus replication on infected cells or the skin lesions may be the result of the host response to the virus. Alternatively, an interaction of viral replication and the host response may produce the lesions. Viruses that replicate in the epidermis, for example, are generally directly responsible for the lesions. Skin lesions of rubella and measles, on

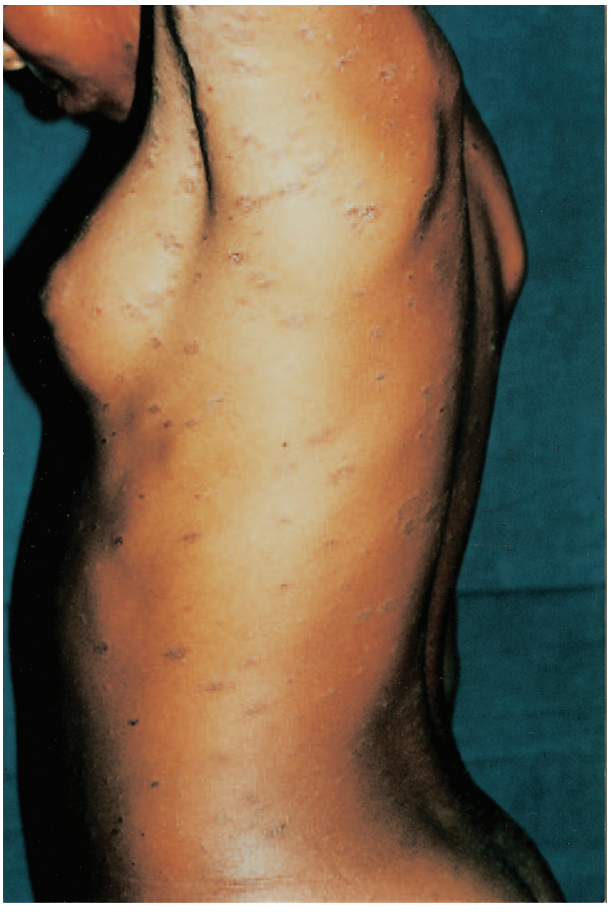


Figure 1.1 Pityriasis rosea.

the other hand, are thought to be at least partly due to the cell-mediated immune response to the virus.

Diagnosis

Confirmation of suspected viral diseases is usually via one of five general methods of laboratory diagnosis: viral cultures, microscopic examination of infected tissue, detection of viral antigens, detection of viral DNA or RNA, or serology. The preferred

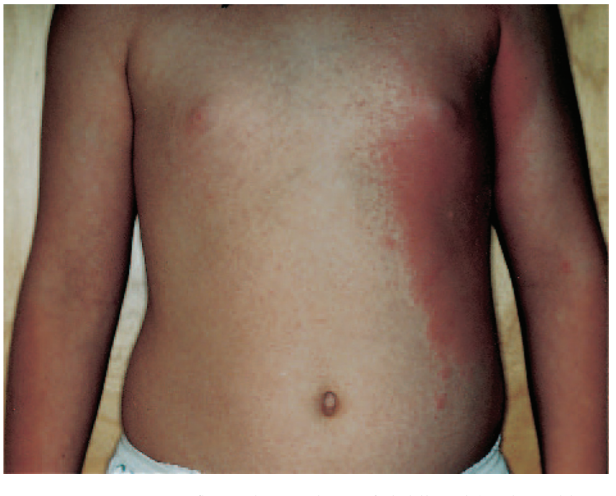


Figure 1.2 Asymmetric periflexural examthem of childhood (unilateral laterothoracic examthem).

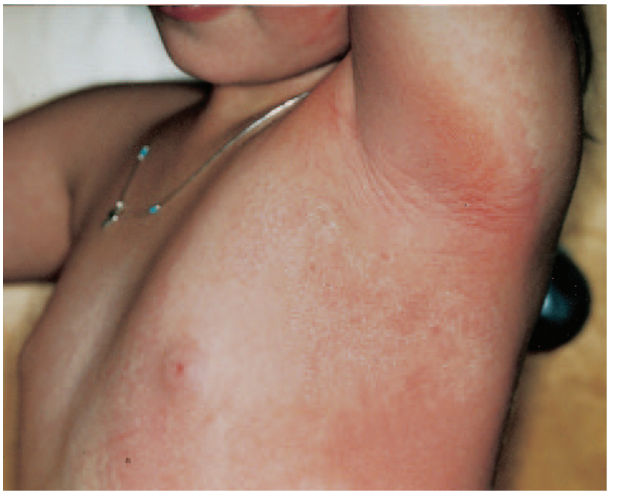


Figure 1.3 Asymmetric periflexural examthem of childhood (unilateral laterothoracic examthem).

method of diagnosis is viral culture when a good culture system is available. A positive culture can be obtained in 1 or 2 days when HSV-1 or -2 is responsible for the lesion. Generally, however, high rates of positivity are seen only when lesions are in the vesicular stage, while later stages of healing are less likely to be positive. Even when fresh vesicular fluid is used to inoculate the appropriate cell culture, positive cultures are more difficult to obtain from VZV.

Papillomaviruses and many other common viral diseases of the skin do not have available culture systems. Microscopic examination of biopsy material can reveal changes consistent with a viral family in such cases, but it is usually not helpful in identifying the specific virus responsible. Histologic changes induced by HPV in benign warts, for example, have similar microscopic appearances. Similar microscopic changes are induced by HSV-1 and -2, as well as by VZV, but are distinctive from changes associated with other herpes viruses. The Tzanck smear is a more rapid procedure than microscopic examination of biopsy tissue to detect changes associated with HSV-1 and -2 and VZV. A smear containing cells scraped from the base of a vesicle is prepared on a glass slide and stained (e.g., with Wright's or Giemsa stain). Multinucleated giant cells will help to confirm that one of the three viruses is responsible for the vesicle, but they cannot specify which virus. Molluscum contagiosum (MC) is another viral infection that can be diagnosed directly from smears from a skin lesion. Intracytoplasmic inclusion bodies (Table 1.2) will help to distinguish papules associated with MC from skin lesions of *Cryptococcus neoformans*, which can appear very similar in HIV-infected patients.

Rapid diagnostic tests for viral antigens are widely available. Fluorescent antibody detection of HSV-1 and -2, as well as VZV, is frequently used in the detection of viral infections of the skin. The three viruses can be distinguished by this technique (in contrast to the Tzanck smear). HPV capsid antigens are sometimes detected by immunoperoxidase techniques, but this technique can be associated with false-negative results with oncogenic HPV types in which viral DNA may be present without capsid antigens. Viral antigens may also be detected by radioimmunoassay or enzyme-linked immunosorbent assay (ELISA). Viral particles or

Table 1.1 Viral Exanthems

Type of rash	Associated virus
Macular/maculopapular	Rubella
	Echovirus (especially 9, 16, 71)
	Coxsackievirus (especially A5, A9, A16, B5)
	Epstein-Barr virus (infectious mononucleosis)
	Human herpesvirus 6 (roseola)
	Rubeola
	Arboviruses (dengue fever)
	Parvovirus B19 (erythema infectiosum)
	Hepatitis B and C
	Human immunodeficiency virus 1
Papular	Human papillomaviruses
	Orf
	Human herpesvirus 8 (Kaposi's sarcoma)
	Milker's nodule
	Molluscum contagiosum
	Human immunodeficiency virus 1
Patches	Epstein Barr virus (oral hairy leukoplakia)
Petechial/purpuric	Coxsackieviruses A5, A9
	Hemorrhagic fever viruses
	Congenital rubella
	Congenital cytomegalovirus
	Echovirus 9
	Epstein-Barr virus
Urticarial	Human immunodeficiency virus 1
	Hepatitis B
	Coxsackieviruses A5, A9
	Epstein-Barr virus
Vesicular/vesiculopustular	Varicella-zoster
	Vaccinia
	Variola
	Herpes simplex virus types 1 and 2
	Coxsackievirus (hand, foot, and mouth disease) (Herpangina)
	Vesicular stomatitis
	Echovirus

viral antigens can also be detected by labor intensive techniques such as electron microscopy or immunoelectron microscopy.

Viruses for which no effective culture system (or serologic assay) is available can be identified by the use of assays to detect viral nucleic acid. HPV is an example, but any virus should be detectable with these methodologies if sufficient knowledge is available regarding the viral genome in order to design specific probes and primers. In situ hybridization is the most widely available technique for detection of viral nucleic acids. Detection of the viral nucleic acid and histologic localization of the virus to specific cells is possible with this technique. Southern hybridization is a more sensitive technique for viral nucleic acid detection and is the basis for greater than 100 HPV types described thus far. The polymerase chain reaction (PCR) is the most sensitive technique for viral nucleic acid detection. A range of viruses within a particular family (i.e., using consensus primers) can be detected or primers used in PCR can be designed to be specific for a particular virus (i.e., type-specific primers). In situ PCR that combines the sensitivity of PCR with specific histologic localization of the virus is even more sophisticated. The Hybrid Capture Assay II is a molecular technique with similar sensitivity as PCR that has become available commercially.

Table 1.2 Viral Inclusion Bodies in Human Diseases

Virus	Location	Eponym
Adenovirus	Nucleus	
Cytomegalovirus	Nucleus, cytoplasm	"Owl's eye"
Herpes simplex (types 1 and 2)	Nucleus	Cowdry type A, Lipschütz body
Measles	Cytoplasm	
Molluscum contagiosum	Cytoplasm	Henderson-Paterson body
Papillomaviruses*	Nucleus, cytoplasm	
Rabies	Cytoplasm	Negri body
Varicella	Nucleus	Cowdry type A
Variola, vaccinia	Cytoplasm	Guarnieri body

*Keratohyaline granules.

Serology provides a fifth technique for diagnosis by using the detection of antibodies elicited by the viral infection. A recent infection is indicated by a four-fold rise in serum antibodies to a specific virus between acute and convalescent sera (usually 4 weeks). A true primary herpetic infection (which would be associated with high levels of immunoglobulin [IgM]) can be distinguished from a first-episode nonprimary infection or a recurrence (i.e., high levels of IgG) by serology. Antibodies to viruses can be detected by a variety of techniques. The responsible virus determines, at least partly, the usefulness of a particular technique. ELISA is considered a screening test for antibodies against HIV, for example. Confirmation with Western blotting must be completed before a definitive diagnosis can be made due to the possibility of a false positive test. Specificity between HSV-1 and -2 antibodies is now adequate with the recently available ELISAs, but detection of IgG against HSV can be made accurately with this test only after 1–4 weeks following primary infection. Antibodies to HSV-1 can be distinguished with sensitivity and specificity from those to HSV-2 using the Western blot.

Differential Diagnosis

A spectrum of nonviral and viral conditions must be considered in the differential diagnosis of various types of viral exanthemata. HSV-1, HSV-2, VZV, poxviruses, hand-foot-mouth viruses, as well as other coxsackieviruses may produce vesicles. During the process of healing, most vesicles develop into pustules. Nonviral entities such as bullous impetigo, insect bite reactions, drug eruptions, contact dermatitis, and gonococcemia must be included in the differential diagnosis of vesiculopustules. Rubella, EBV infections (i.e., infectious mononucleosis), HHV-6 infection (i.e., roseola), as well as a variety of coxsackievirus (A and B) and echovirus infections may produce macules. Drug eruptions and bacterial infections (e.g., scarlet fever, Rocky Mountain spotted fever, erysipelas) are possible nonviral etiologies of macules. Measles, echovirus infections, and human parvovirus B19 infections (i.e., erythema infectiosum) may result in macules presenting with papules. Any of the macular or papular nonviral conditions noted above, as well as erythema multiforme, which is commonly of viral etiology (i.e., HSV), may produce maculopapular lesions or they may be associated with nonviral infections or with drug eruptions. A spectrum of poxvirus and HPV infections, as well as in Gianotti-Crosti syndrome, which may be a manifestation of hepatitis B or a variety of other viral infections, may manifest as papules. Bacterial infections (e.g., Bartonella, Mycobacterium), fungal infections (e.g., cryptococcus), and noninfectious

conditions (e.g., seborrheic keratoses, basal cell carcinomas) may also be papules. Poxvirus infections (e.g., orf, milker's nodules), HPV (e.g., squamous cell carcinomas associated with HPV-16), or herpesvirus 8 (e.g., Kaposi's sarcoma), mycobacterial and Bartonella infections (e.g., bacillary angiomatosis), and noninfectious tumors (e.g., basal cell carcinomas, squamous cell carcinoma, melanoma, pyogenic granuloma) may be nodular. Allergic reactions, including drug eruptions, as well as hepatitis B or coxsackie A9 virus infections, are usually associated with urticaria. Dengue fever and other hemorrhagic fevers (e.g., Lassa fever) may result in petechiae, but this finding may occur in nonviral conditions producing thrombocytopenia. Viral infections such as HSV-1, HSV-2, VZV, CMV, and hand-foot-mouth disease (HFMD) commonly cause ulcerations of the mucous membranes. Immunocompromised persons sometimes suffer oral or anogenital ulcers due to CMV, or such ulcers may involve a coinfection of CMV and HSV. Nonviral ulcers such as aphthous stomatitis must be distinguished from oral ulcers of viral etiology. Stasis dermatitis or other causes of decreased circulation may cause cutaneous ulcers.

DNA VIRUSES

Poxviruses

Poxviruses are large DNA viruses that are members of the family Poxviridae; those of clinical significance include smallpox, vaccinia, MC, orf, and milker's nodules (Table 1.3). The only one of these viruses with significant mortality, smallpox, has been eradicated via worldwide vaccination programs that resulted in the last patient with epidemic smallpox being treated in 1977 [1].

Smallpox

Although smallpox replicates in the epidermis, it is spread not only via direct skin contact and fomites, but also by respiratory transmission. Patients experience 3 days of apprehension, preceding development of skin lesions; this is followed by sudden prostrating fever, severe headache, back pain, and vomiting. Tense, deep-seated papules and vesicles are preceded by erythematous macules. Pustules follow the vesicles, then crusts, and finally scar formation. All lesions are in the same stage of development with the rash appearing in a centrifugal distribution. The hemorrhagic form of smallpox results in almost 100% mortality even before development of skin lesions, although the overall mortality rate with smallpox is approximately 30%.

Vaccinia

Vaccination against the vaccinia virus is no longer routinely used since smallpox has been eradicated (Tables 1.4 and 1.5). While use of the vaccinia virus to immunize against smallpox was one of the greatest success stories in medical history, use of this live virus occasionally led to complications in susceptible individuals, such as bacterial superinfection, abnormal viral replication, or altered reactivity [2].

Molluscum contagiosum

The most prevalent poxvirus is molluscum contagiosum (MC); the incubation period of MC is 2–7 weeks. MC presents as 3 to 6-mm skin-colored papules with a central umbilication. While two different strains of MC (I and II) have been identified (based on restriction endonuclease digestion patterns), both strains produce similar clinical pictures. MC often follows one of two patterns of clinical presentation in immunocompetent individuals: widespread papules on the trunk and face of children transmitted by direct skin-to-skin (nonsexual) contact or genital papules in adults spread by sexual contact. In either case, it is unusual to see more than 20 lesions per patient [3]. In immunocompromised persons, especially those who are HIV positive, MC can present with thousands of papules and be a major source of morbidity; prevalence rates in this population range from 9 to 18% [4].

Orf

Contagious ecthyma, orf, is a less common poxvirus that is transmitted from sheep, goats, etc., to the hands of humans. The cutaneous presentation of orf is usually nodules averaging 1.6 cm in diameter associated with regional lymphadenopathy, lymphangitis, and fever. Orf lesions spontaneously progress through six stages, resulting in healing in about 35 days [5].

Milker's nodules

A paravaccinia virus causes milker's nodules, which is similar to orf except that lesions result from manual contact with teats of infected cows, and milker's nodules have an incubation period of 4–7 days. The nodules heal in 4–6 weeks after progressing through six clinical stages similar to orf [6].

Primary viremia follows local multiplication in the respiratory mucosa and regional lymphoid tissue after contact with smallpox via the respiratory route. A secondary viremia is associated with the initiation of the prodrome after spreading throughout the reticuloendothelial system. Thrombocytopenia accompanies the development of skin lesions which, in hemorrhagic forms of smallpox, can become severe and result in disseminated intravascular coagulation with decreases in accelerator globulin, prothrombin, and proconvertin, ending with extensive hemorrhage and death.

Regional lymphadenopathy sometimes accompanies a local reaction to vaccinia replication in the epithelium. The host immune response limits systemic manifestations except in cases of depressed immunity or in diseases with inadequate epithelial barriers. Generalized vaccinia can result, but it is rarely a lethal disease like smallpox. Viral replication in MC, orf, and milker's nodules is generally limited to the epidermis, but dermal changes are also seen in milker's nodules.

Pathology. Lesional biopsy of smallpox reveals cytoplasmic eosinophilic inclusion bodies (Guarnieri bodies) along with papules, vesicles, or pustules. Electron microscopy or fluorescent antibody staining can identify smallpox, or the virus can be isolated with appropriate tissue culture systems. A history of vaccination along with the clinical presentation is usually sufficient for diagnosis of vaccinia, but diagnostic tools similar to those used for smallpox may be used to detect this virus.

Histologically, a hypertrophied and hyperplastic epidermis overlying a normal-appearing basal layer characterizes MC. Multiple Feulgen-positive intracytoplasmic inclusion bodies (Henderson Paterson bodies or molluscum bodies) are seen in the enlarged epidermal cells.

Laboratory Findings. Laboratory findings with orf and milker's nodules, as with MC, are generally limited to histology. The histopathology varies with the clinical stage in the case of the latter

Table 1.3 Taxonomy of Human Viruses

Family	Subfamily, genus	Type species or example	Morphology	Envelope	Chapter
DNA VIRUSES					
dsDNA viruses					
Poxviridae			Ovoid	+	3
	Chordopoxvirinae				
	Orthopoxvirus	Vaccinia virus, variola			
	Parapoxvirus	Orf virus			
	Molluscipoxvirus	Molluscum contagiosum virus			
	Yatapoxvirus	Yaba monkey tumor virus			
Herpesviridae			Icosahedral	+	
	Alphaherpesvirinae				
	Simplexvirus	Human herpesviruses (HSV) 1 and 2			4
		Cercopithecine herpesvirus 1 (herpesvirus B)			11
	Varicellovirus	Human herpesvirus 3 (VZV)			5
	Betaherpesvirinae				
	Cytomegalovirus	Human herpesvirus 5 (CMV)			7
	Roseolovirus	Human herpesvirus 6 and 7[a]			8,9
	Gammaherpesvirinae				
	Lymphocryptovirus	Human herpesvirus 4 (EBV)			6
	Rhadinovirus	(HHV-8)			10
Adenoviridae	*Mastadenovirus*	Human adenoviruses	Icosahedral	−	27
Papovaviridae	*Polyomavirus*	JC virus, Merkel cell polyomavirus	Icosahedral	−	
Papillomaviridae	*Papillomavirus*	Human papillomaviruses	Icosahedral	−	12
SsDNA viruses					
Parvoviridae	*Parvovirinae*				
	Erythrovirus	B19 virus			
	Dependovirus	Adeno-associated virus 2[a]			13
	Bocavirus	Human Bocavirus			
Circoviridae	*Circovirus*	TTB	Icosahedral		
DNA and RNA reverse transcribing viruses					
Hepadnaviridae	*Orthohepadnavirus*	Hepatitis B virus	Icosahedral	−	24
Retroviridae			Spherical	+	
	Deltaretroviruses	HTLV-I and II			15
	Lentivirus	Human immunodeficiency viruses			14
	Spumavirus	Human spumavirus[a]			
RNA VIRUSES					
DsRNA viruses					
Reoviridae			Icosahedral	−	
	Orthoreovirus	Reovirus 3[a]			
	Orbivirus	Kemerovo viruses			
	Rotavirus	Human rotaviruses			
	Coltivirus	Colorado tick fever virus			16
	Seadornavirus	Banna virus			
Negative Stranded SsRNA viruses					
Paramyxoviridae			Spherical	+	
	Paramyxovirinae				
	Respirovirus	Human parainfluenza viruses			
	Morbillivirus	Measles virus			
	Rubulavirus	Mumps virus			17
	Henipavirus	Nipah virus			
	Pneumovirinae	Human respiratory syncytial virus			
	Pneumovirus				
	Metapneumovirus	Human metapneumovirus			
Rhabdoviridae			Bacilliform	+	
	Vesiculovirus	Vesicular stomatitis virus			
	Lyssavirus	Rabies virus			
Filoviridae	*Filovirus*	Ebola virus, Marburg virus	Bacilliform	+	18
Orthomyxoviridae			Spherical	+	
	Influenzavirus A	Influenza A virus			
	Influenzavirus B	Influenza B virus			
	Influenzavirus C	Influenza C virus			

(*Continued*)

Table 1.3 (Continued)

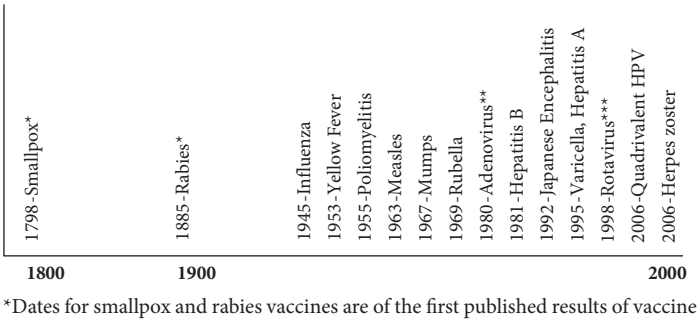
Family	Subfamily, genus	Type species or example	Morphology	Envelope	Chapter
Bunyaviridae			Amorphic	+	19
	Orthobunyavirus	Bunyamwera virus, LaCrosse virus			
	Hantavirus	Hantaan virus, Sin Nombre virus			
	Nairovirus	Crimean-Congo hemorrhagic fever virus			
	Phlebovirus	Rift Valley fever virus			
Arenaviridae			Spherical	+	
	Arenavirus	Lymphocytic choriomeningitis virus, Lassa fever virus, South American hemorrhagic fever viruses			20
Positive stranded ssRNA virus			Icosahedral	–	
Picornaviridae	*Enterovirus*	Polioviruses, Coxsackieviruses, Echovirus			21
	Rhinovirus	Human rhinoviruses			
	Hepatovirus	Hepatitis A virus			24
Caliciviridae	*Calicivirus*	Norwalk virus	Icosahedral	–	
Hepeviridae	*Hepevirus*	Hepatitis E		+	24
Astroviridae	*Astrovirus*	Human astrovirus 1	Icosahedral		
Coronaviridae	*Coronavirus*	Human coronavirus	Pleomorphic	+	
Flaviridae	*Flavivirus*	Yellow fever virus, Dengue virus	Spherical		22
	Hepacivirus	Hepatitis C virus			24
Togaviridae	*Alphavirus*	Western equine encephalitis virus, Chikungunya	Spherical		23
	Rubivirus	Rubella virus			
Subviral Agents: Satellites, Viroids, and Agents of Spongiform Encephalopathies					
Satellites (single-stranded RNA)	*Deltavirus*	Hepatitis delta (D) virus	Spherical	–	
Prion protein agents		Creutzfeld-Jakob agent	?	–	25

[a]Human virus with no recognized human disease.

two diseases. In the early stages, both intracytoplasmic and intranuclear inclusions may be observed.

Management. The only effective management of smallpox proved to be prevention via vaccination. Management of symptoms and prevention of bacterial superinfection were paramount for patients with smallpox or disseminated vaccinia. Thiosemicarbezone and antivariola or antivaccinia sera had limited effectiveness (Table 1.6). Liquid nitrogen, curetting, imiquimod or cidofovir can be used to treat MC. In immunocompromised persons, recurrences are common. Excision and cautery can remove lesions of orf or milker's nodules, but this is usually not necessary as spontaneous resolution can be expected in approximately 6 weeks.

Table 1.4 Timeline of Virus Vaccine Development*



*Dates for smallpox and rabies vaccines are of the first published results of vaccine usage. Remaining dates are of FDA approval of a vaccine.
**No longer available.
***Subsequently replaced by two new rotavirus vaccines.

Human Papillomaviruses

HPVs are nonenveloped, double-stranded DNA viruses that belong to the family Papillomaviridae. Regional tropism (i.e., whether they produce genitomucosal lesions, nongenital lesions in the general population, or lesions associated with epidermodyplasia verruciformis [EV]) can be used to categorize HPV. Location of the lesions, quantity of HPV in the lesion, degree and nature of the contact, and immune status of the exposed individual determine the transmission of HPV. Genital (venereal) warts, condyloma acuminatum, are the most prevalent clinical form of viral genitomucosal lesions; the incidence of these warts has risen six-fold during the past three decades. Classification of HPVs also may be according to their malignant potential. More than 90% of condyloma acuminatum are clinically benign and are due to HPV-6 or HPV-11. HPV types 31, 33, and 35 have intermediate malignant potential. By contrast, HPV types 16 and 18 have high malignant potential; over 70% of cervical and other anogenital cancers contain DNA from one of the latter two types [7–9]. Orogenital sex can transmit these HPV types to other mucous membranes resulting in oral condyloma acuminatum, or HPV may be transmitted nonsexually such as during vaginal delivery [10]. HPV from vaginal warts in the latter case may be transmitted to the oral or respiratory tract of the infant and present as respiratory (laryngeal) papillomas [11]. As a result of HPV acquired during vaginal delivery, anogenital warts may also develop in infants within a few months of birth. While sexual abuse can produce anogenital warts in children, a significant proportion of such warts results from incidental spread from cutaneous warts [12]. Oral warts not of genital origin can be seen in focal epithelial hyperplasia that

Table 1.5 Virus Vaccines: Recommendations for Administration*

Vaccine	Target population	Route	Dosage	Comments
MMR (measles, mumps, rubella)	Children	SC	2 doses at 12–15 mos and 4–6 yrs	Also available: Measles and Rubella (live): MRVAX II® Measles (live attenuated): ATTENUVAX® Mumps (live): MUMPSVAX Rubella (live): MERUVAX Rubella + Mumps (live): BIAVAX II
Varicella Zoster Varivax®	Children and susceptible adults	SC	2 doses: at ages 12–15 mos and at 4–6 yrs 2 doses (4–8 wks apart in susceptible persons ≥ 13 yrs	Proquad (MMR +Varivax)
Zostavax	Adults >60 years	SC	1 dose	
Influenza Flumist, FluLaval, Afluria, Fluarix Fluzone® Fluvirin® Fluogen® Flushield®	Persons ≥65 yrs, residents of chronic care facilities, those with chronic cardiopulmonary diseases, or those who may transmit the virus to high-risk persons	IM	6 mos – 8 yrs: 1 or 2 doses of split virus only, at least 1 month apart 9–12 yrs: 1 dose of split virus only >12 yrs: 1 dose of whole or split virus	Vaccinate from September to November Flumist is a live attenuated vaccine given intranasally
Hepatitis A	Children and susceptible adults	IM	2 doses: Havrix® - >2 yrs: 0 mos & 6–12 mos Vaqta® - 2–17 yrs: 0 mos & 6–18 mos >17 yrs: 0 mos & 6 mos	Havrix® is available in combination with Engerix-B® as Twinrix®
Hepatitis B Recombivax HB® Engerix B® Comvax®(with haemophilus influenza type B vaccine)	Children and susceptible adults	IM	3 doses: Infants: birth to 2 mos, 1–4 mos & 6–18 mos Children and adolescents: mos 0, 2, & 4 Adults: mos 0, 1, & 6	Infants with HbsAG-positive mothers receive 1st dose within 12 hours of birth. 2nd dose at 1 month, and 3rd dose at 6 months. Engerix-B® is now available in combination with Havrix® as Twinrix®.
Rabies Imovax, Rabavert **RABIE-VAX®**	Persons at risk of rabies exposure or those recently exposed		Preexposure: 3 doses on days 0, 7, & 21 or 28 IM HDCD: Imovax® IM PCEC: RabAvert™ IM Rabies Vaccine Absorbed ID HDCD: Imovax® Rabies ID Postexposure: 5 doses on days 9, 3, 7, 14, & 28 IM HDCV IM Rabies Vaccine Absorbed IM PCEC	Previously vaccinated persons require only 2 doses after rabies exposure, on days 0 & 3.
Poliomyelitis Poliovax® IPOL® Orimune® (live, oral)	Children	SC Oral	Sequential series: 4 doses 2 mos: IPC (inactivated polio vaccine) 4 mos: IPV 6–18 mos: OPV (oral polio vaccine) 4–6 yrs: OPV	Regimens with all IPV or all OPV are given in the same time frame. All IPV doses are indicated for immunosuppressed patients or contacts. All OPV dosing is accepted in certain circumstances only.
Yellow Fever YF-VAX®	Persons traveling to endemic countries (parts of Africa and South America)	SC	1 dose	Booster given every 10 years for recertification for travel into endemic countries
Japanese Encephalitis Ixiaro JE-VAX®	Persons traveling to certain parts of Asia	SC	3 doses, on days 0, 7, & 30	
Rotavirus Rotarix RotaTeq	Children	Oral		
Human Papillomaviruses types 6, 11, 16, 18 Gardasil	Females from 9 to 26 years	IM	3 doses on months 0, 2 & 6	Virus-like particle (VLP) vaccine made from recombinant L-1 major capsid proteins

IM=intramuscular; SC=subcutaneous; ID=intradermal; mos=months; yrs=years; HDCV=human diploid cell vaccine; PCEC=purified chick embryo cell culture vaccine.

*Vaccinia vaccine for prevention of smallpox not generally available (or recommended) and when available, limited to certain military forces.

Table 1.6 Immunoglobulins (IG): Indications for Administration*

Immunoglobulin	Generic/trade name	Approved indication
Intramuscular IG	BAYGAM	Exposure to measles or hepatitis A in susceptible persons; varicella (if VZIG unavailable); rubella
Hepatitis B-IG	BAYHEPB, NABI-HB, HYPER HEP	Hepatitis B exposure in susceptible persons
Human rabies IG	BAYRAB, IMOGAM-Rabies, HYPERAB	Rabies exposure in previously unvaccinated persons
Varicella-Zoster IG	VZIG	Susceptible persons exposed to varicella who have a high risk for complications (e.g. immunocompromised patients and neonates)
Respiratory syncytial virus IG	Respigam® (Palivizumab) Synagis®	Prophylaxis in high-risk infants (e.g. those with bronchopulmonary dysplasia or prematurity)
Cytomegalovirus	Cytogam	CMV prophylaxis in seronegative renal transplant recipients of a kidney from a CMV-positive donor

*Vaccinia immune globulin generally not available, and when available, limited to certain military forces.

contains such unique HPV types as 13 or 32 and they present most commonly in certain ethnic groups [13]. In the general population, cutaneous warts are very common and can present as verruca vulgaris (HPV-2), plantar warts (HPV-1), or verruca plana (HPV-3). These verrucous papules rarely lead to major medical problems, but can be annoying and difficult to eradicate.

On the other hand, cutaneous warts in EV can lead to major morbidity and mortality [14]. EV was the first model of cutaneous viral oncogenesis in humans and is a rare condition that can occur sporadically or in an autosomal recessive manner. During childhood, disseminated warty papules and erythematous macules develop in EV patients. Approximately one-half of these patients will develop cutaneous carcinomas in adulthood. EV is associated with at least 17 HPV types. HPV-3 and -10 are also found in flat warts in the general population, but most are unique to EV. Malignant transformation in EV is mostly associated with HPV-5 and -8. Oncogenic HPV in EV appears to be necessary but not sufficient for malignant transformation, which is analogous to the situation with HPV-16 and -18 in anogenital cancers in the general population. In both cases, cofactors appear to be necessary. Cofactors, including cigarette smoking, other transactivating viruses, genetics, and diet, may be important in anogenital malignancies, but the individual role of each cofactor is not clear [15]. The most important cofactor in EV is ultraviolet irradiation, which is illustrated by the fact that the highest incidence of carcinomas in EV patients is in areas of greatest sunlight exposure [16].

HPV DNA replication, RNA transcription, and late protein production are coordinated by the state of differentiation of the epithelial cell following infection of the basal layer of the epidermis. Early (E) proteins direct viral replication. Late (L) proteins, L1 and L2, are viral capsids, which are synthesized and assembled into virions in the nuclei of the granular layer [17]. Verruca are produced in approximately 2–9 months, but HPV DNA can remain in a latent state in normal-appearing skin or mucous membranes for much longer periods of time. Therefore, in some cases, the incubation time from infection to lesion development may be years.

Since newer warts tend to have more virions than do older verrucae, the copy number of HPV DNA varies according to the age of the lesions. Plantar warts usually have more virions than do condyloma acuminatum, and benign warts have more virions than do dysplastic or neoplastic HPV-related lesions.

Pathology. If the lesion is assumed to be HPV related and benign, often no laboratory tests are carried out. The following general patterns may be observed in tissues from biopsies of verrucae: acanthosis, papillomatosis, hyperkeratosis, parakeratosis, and prominent and often thrombosed dermal capillary vessels. Often such features as koilocytes, large keratinocytes with an eccentric, pyknotic nucleus surrounded by a perinuclear halo, are observed. A biopsy is sometimes taken to determine if the lesion is dysplastic or neoplastic. Such biopsies would most likely be taken in the anogenital region in the general population. Dysplastic or neoplastic lesions are most frequent on the cervix and would be detectable via cytopathology taken with the Papanicolaou smear.

Laboratory Findings. Immunohistochemical staining of HPV capsid antigens for more specific detection of HPV can be done. This method may give false-negative results with such lesions since dysplastic or neoplastic lesions contain few, if any, capsid antigens. The only specific method of diagnosing HPV is via DNA detection methods, since HPV cannot be readily grown in tissue culture nor is serology routinely available. Over 100 HPV types are recognized based on Southern hybridization [18]. A new HPV genotype is designated if the virus differs more than 10% in nucleotide sequence in the "late gene" L1 open reading frame from previously identified HPV types. Although in situ hybridization and Hybrid Capture Assay II have become widely available, detection of specific HPV types is more frequently a research tool than a routine laboratory procedure. Hybrid Capture Assay II and PCR, however, are by far the most sensitive methods of detecting HPV DNA [19].

Management. Treatment for most benign verrucae includes surgery, cryotherapy, or topical chemotherapy. The objective in each case is to eradicate the lesion and allow the immune system to hold latent HPV in surrounding (normal-appearing) tissue in check to prevent recurrences. Simple excision, electrodesiccation, and removal with a CO_2 laser are all types of surgical therapy. Liquid nitrogen for destruction of the lesion is a form of cryotherapy. Podophyllin resin, purified podophyllotoxin, 5-fluorouracil, retinoic acid, cantharidin, salicylic acid, lactic acid, sinecatechins ointment, bichloroacetic acid, and trichloroacetic acid are options for topical chemotherapy [20–22]. The size and location of the wart, as well as the history of previous therapies, are determinants in selection of the most appropriate therapy.

Interferon (IFN-α) for treatment of condyloma acuminatum is the only antiviral therapy approved for HPV (Table 1.7) [23].

Table 1.7 FDA-approved Anti-HPV Agents

Generic name	Trade name
Interferon-α	Roferon A
	Intron A
	Alferon
Imiquimod	Aldara*

*Approved as an immune response modifier since the antiviral activity is indirect. Veregen (sinecatechins) also approved, but mechanism of action unknown.

Although IFN-α is effective in eradicating 50–70% of genital warts, its most effective use is in combination therapy [24]. IFN-α given subcutaneously following laser excision of condyloma acuminatum, for example, markedly reduces the recurrence rate. Surgery is used for therapy of HPV-related malignant lesions; if metastases are present, chemotherapy is usually included. Antisense oligonucleotides and cidofovir (a broad-spectrum agent active against a variety of DNA viruses) are new treatments for HPV-related lesions currently under study [25,26]. An immunomodulatory agent demonstrated to be very effective for condyloma acuminatum is imiquimod [27]. The patient applies imiquimod topically and it produces minimal local inflammation and no systemic side effects. The mode of action of imiquimod is via induction of endogenous IFN-α as well as a wide variety of other cytokines. Recurrence rates following clearance of lesions with imiquimod are very low. HPV vaccines containing recombinant L-1 major capsid proteins in virus-like particles (VLPs) are approved for the prophylaxis of anogenital lesions due to HPV 6, 11, 16, and 18.

Human Herpesviruses

The family Herpesviridae is composed of double-stranded DNA viruses and is divided into three subfamilies: alphaherpesviruses (HSV-1, HSV-2, VZV, and B virus); betaherpesviruses (CMV, HHV-6, HHV-7), and gammaherpesviruses (EBV, HHV-8). Primary VZV reaches the skin as a result of a secondary viremia, but primary HSV infects the skin via direct inoculation. The skin is infected by local spread from an internal focus (i.e., the nerve) in recurrent HSV and VZV. Viral replication at nonepithelial sites can result in infrequent skin lesions secondary to EBV or CMV. Although HHV-6 infection frequently produces an exanthem, viral replication is occurring in the peripheral blood mononuclear cells (especially T cells). While no specific disease has yet been proved to be due to HHV-7 infection, PR has been closely associated with this virus. HHV-8 has been identified both in the endothelial cells of Kaposi's sarcoma and the epithelial cells of squamous cell carcinomas of organ transplant patients, but its role in the etiology of the latter tumor is not clear. Herpesvirus simiae, an animal herpes virus (B virus), can also cause human disease, most significantly a fatal encephalomyelitis. The virus can be recovered from vesicular skin lesions at the point of inoculation as well as from vesicles possibly arising from reactivation of latent B virus infection.

Herpes Simplex Viruses 1 and 2

Although most known for causing cold sores and genital herpes, respectively, HSV-1 and HSV-2 cause several other mucocutaneous infections, such as gingivostomatitis, herpes gladiatorum, eczema herpeticum, herpes whitlow, neonatal herpes, lumbosacral herpes, herpetic keratoconjunctivitis, and herpes encephalitis. Erythema multiforme is usually caused by HSV. These viruses typically cause a primary mucocutaneous infection followed by a latent infection when the virus remains dormant in the neuronal ganglia. Viral reactivation and movement down the nerve to produce active mucocutaneous infections is seen with recurrent disease.

Three to fourteen days following sexual exposure to an infected partner, primary genital herpes may occur. In most transmissions, the source partner may be shedding HSV asymptomatically. Widespread genital vesicles and ulcers, edema, pain, inguinal lymphadenopathy, discharge, dysuria, malaise, fever, photophobia, and occasionally aseptic meningitis can be seen during the primary episode. The severity of these signs and symptoms is usually greater in women than in men; 3–4 weeks are often required for complete healing. Viral shedding lasts up to approximately 10 days in men and 14 days in women [28], but can recur asymptomatically at any time.

The first recognized episode of genital HSV, however, is often not truly primary. This first clinical manifestation of a virus that has remained latent in the infected nerve for an extended period of time (i.e., months or even years) would be considered a first episode, nonprimary outbreak. In such cases, signs and symptoms are usually less severe than in true primary genital HSV and may require only 2–3 weeks for complete healing. The pre-existence of sufficient levels of IgG to attenuate the disease is the major reason for the decreased severity of first episode, nonprimary genital herpes.

An increasing proportion (e.g., 30%) of first-episode genital herpes is due to HSV-1, which is often attributable to orogenital contact. Outbreaks of genital herpes due to HSV-1, however, are usually less severe than those due to HSV-2.

While at least 45 million individuals in the USA are estimated to be seropositive for HSV-2, approximately 11 million persons have recognized recurrent genital herpes [29–37]. According to one study, approximately one-half of seropositive persons who deny a history of genital herpes can be taught to recognize signs and symptoms of the disease. Another investigation demonstrated that the majority of persons seropositive for HSV-2 via Western blotting shed the virus asymptomatically at least occasionally. Virus traveling down the sensory nerve first causes prodromal sensations of pruritus or tingling followed shortly by the formation of vesicles. This is the result of reactivation of HSV, which lies dormant in neuronal ganglia. A variety of factors, such as emotional or physical stress (e.g., menstrual periods) or mild trauma (e.g., sexual intercourse), may trigger recurrences. HSV recurrent episodes are usually less severe than initial outbreaks and often heal in 7–10 days without therapy. Genital herpes due to HSV-2 recurs more frequently than HSV-1-associated disease. Compared to men, women suffer 20% less recurrences of genital herpes, a factor that may contribute to the higher rate of herpes transmission from men to women than from women to men. HSV recurrences in immunocompromised patients may be chronic and result in large ulcerations if not treated.

HSV-1 is associated with greater than 90% of orolabial herpes, which is usually acquired early in life [32,33]. Primary HSV-1 infection may present as acute gingivostomatitis with a peak

incidence between the ages of 1 and 5 years. Five to ten days after exposure to HSV, primary gingivostomatitis often presents with sore throat, regional lymphadenopathy, fever, and widespread painful ulcerations of the oral cavity and lips.

Up to 90% of adults in various seroepidemiologic surveys have serologic evidence of HSV-1 infection [33]. Recurrent herpes labialis, however, is seen in 20–40% of the population. The majority of orolabial herpes infections remain asymptomatic, analogous to the situation with genital herpes. In certain susceptible individuals, not only stress and trauma, but also exposure to sufficient ultraviolet light, can induce recurrent episodes of herpes labialis. Erythema and vesicle formation are preceded by a few hours of prodromal symptoms of pruritus, tingling, and pain. Formation of vesicles usually occurs on the vermilion border of the lip, but occasionally may be seen around the lips. Such lesions contain culturable HSV for approximately 4 days. During the next 10 days, vesicles ulcerate, crust, and usually undergo complete healing.

Varicella Zoster Virus

The presentation of VZV can be as primary varicella (chickenpox) or as the recurrent form, herpes zoster (shingles) [34]. Children usually develop primary varicella, which presents with the simultaneous onset of rash, low-grade fever, and malaise. The exanthem is often preceded by up to 3 days of prodromal symptoms in older children and adults, including headache, myalgia, anorexia, nausea, and vomiting. The face and trunk first develop lesions that appear as erythematous macules and rapidly progress over the next 12–14 hours to papules, vesicles, pustules, and crusts. Most skin lesions are seen on the trunk and on proximal extremities. Pruritus is the most prevalent symptom. Varicella is characterized by the simultaneous presence of lesions in all stages of development in the same anatomic region due to the rapid evolution of successive crops of lesions. Shallow, painful ulcers develop from rapid erosion of vesicles that appear on mucous membranes. Scarring, which may be due to bacterial secondary infection, is the most common cutaneous complication of varicella in immunocompetent persons. Significant morbidity and occasional mortality can result from such complications as central nervous system (CNS) involvement, varicella pneumonia, or varicella hepatitis in adults and in immunocompromised individuals. A 2% risk of congenital malformations is associated with maternal varicella if infection occurs during the first 20 weeks of pregnancy.

VZV persisting in sensory ganglia reactivates, usually after many years, in 20% of immunocompetent persons and in 20–50% of immunocompromised patients. This reactivation causes a transient viremia and spreads down the sensory nerve, producing radiculoneuritis [35]. Vesicles appear along the distribution of the sensory nerve after a few days (to weeks) of pain. Fever, regional lymphadenopathy, malaise, and, occasionally, a flu-like syndrome can be associated with pain. It is not unusual for a few lesions to appear in neighboring dermatomes, although vesicles generally occur only along one dermatome. The areas usually affected most severely by primary varicella are those same anatomic regions (i.e., face and trunk) that have the greatest predilection for zoster. Pustules result after a few days when the vesicles are infiltrated by leukocytes. Pustules begin to dry after 1–2 weeks, resulting in crusts that are usually lost by 1 month after the appearance of the first vesicle.

Cutaneous complications are rare, although scarring can occur, particularly in darker skinned individuals [36]. Postherpetic neuralgia, which can be defined as any pain remaining after full cutaneous healing, is the most prevalent complication. The pain can be extremely severe, can be treatment resistant, and can last months to years. Disseminated herpes zoster, defined as more than 20 vesicles outside the primary and adjacent dermatomes, is rare in normal hosts; but severely immunocompromised patients have a risk of dissemination approaching 40%. Cutaneous dissemination can herald significant morbidity and mortality, because it may be a marker of visceral involvement (i.e., liver, lungs, CNS).

Vision impairment or blindness with involvement of the ophthalmic branch of the trigeminal nerve is another complication of herpes zoster, not uncommonly seen in normal hosts [37]. Involvement of the facial and auditory nerves can result in the Ramsey-Hunt syndrome. CNS involvement and motor paralysis less commonly can result from herpes zoster [38,39].

Cytomegalovirus

The seroprevalence for CMV increases with age such that most adolescents are seropositive and nearly 100% of older individuals are CMV seropositive. In immunocompetent persons, primary infection is asymptomatic and usually subclinical. In most normal hosts, the virus remains latent. However, it can produce clinical symptoms in neonates and in immunocompromised persons, but skin involvement is rare. During pregnancy, primary CMV infection results in intrauterine infection in 55% of fetuses. If infection occurs during the first trimester, sequelae are most severe [40]. Infection with CMV is the major infectious cause of mental retardation and deafness in the USA and is the most common congenital viral infection. CMV can produce purpuric macules and papules due to persistent dermal hematopoiesis, like other causes of the TORCH syndrome (i.e., toxoplasmosis, other [syphilis/bacterial sepsis], rubella, CMV, HSV), resulting in the clinical picture termed blueberry muffin baby [41]. A variety of skin lesions, from vesicles to verrucous plaques, have been reported in association with immunocompromised patients. The most prevalent cutaneous manifestation of CMV is ulceration, especially in the perianal area [42,43].

Epstein-Barr Virus

In immunocompetent individuals, the most prevalent clinical manifestation of EBV infection is infectious mononucleosis [44]. In infectious mononucleosis, the incubation period of EBV is 30–50 days followed by a prodrome characterized by malaise, headache, and fatigue followed by fever, sore throat, and cervical adenopathy. Small petechiae are observed at the border of the hard and soft palate in approximately one-third of patients. Cutaneous manifestations of infectious mononucleosis, such as macules or papules and, less commonly, erythema, vesicles, petechiae, or purpura, occur in 3–16% of patients [45]. These lesions are more common on the trunk and upper arms, last 1–7 days, and present during the first week of illness. A high percentage of patients develop erythematous macules and papules over the trunk and extremities after approximately 1 week if ampicillin or certain other penicillins are given to a person with infectious mononucleosis [46]. After about 1 week, these lesions are followed by desquamation.

Table 1.8 The Classic Childhood Exanthems (Named in Early 1900s)

First Disease:	Rubeola (Measles)
Second Disease:	Scarlet Fever
Third Disease:	Rubella
Fourth Disease:	Filatov-Dukes (staphylococcus scalded skin syndrome?)
Fifth Disease:	Erythema Infectiosum
Sixth Disease:	Exanthem Subitum (Roseola Infantum)

The epithelial cells of oral hairy leukoplakia, an oral lesion closely associated with HIV infection, also contain EBV DNA [47,48]. In addition, B-cell lymphomas of immunocompromised individuals often produce mucocutaneous lesions and contain EBV DNA. Symmetric, nonpruritic, lichenoid papules of the face, limbs, and buttocks, known as Gianotti-Crosti syndrome, have also been associated with primary EBV infection [49].

Human Herpesvirus Type 6

HHV-6 is presently recognized as the cause of exanthem subitum (roseola infantum), which was also termed sixth disease long before HHV-6 was isolated (Table 1.8) [50]. Exanthem subitum usually occurs after an incubation period of 5–15 days, in infants from 6 months to 2 years of age, with high fever lasting 3–5 days. The infant may not appear in distress despite the high fever, but can have such signs as palpebral edema, inflammation of the pharynx, and lesions of the soft palate. A macular to papular eruption appears on the trunk and neck as the fever resolves. Manifestations of the disease, including the rash, usually fade in 1–2 days without treatment [51–53].

Human Herpesvirus Type 7

HHV-7 has been associated with certain cases of roseola infantum as well as with PR, but it is not currently proved to be the etiologic factor in any disease.

Human Herpesvirus Type 8

HHV-8 has been detected both in Kaposi's sarcoma from HIV-infected persons as well as classic (HIV-negative) Kaposi's sarcoma and was termed Kaposi's sarcoma-related herpesvirus [54–57]. The same viral sequences have been reported from squamous cell carcinomas and other epithelial lesions from patients with organ transplants [58]. The role of HHV-8 in epithelial tumors is unknown, however, this virus is considered necessary, but not sufficient, to cause Kaposi's sarcoma. Cofactors for development of this tumor are under study.

B Virus (Herpesvirus simiae)

Many nonhuman herpesviruses exist, but B virus is of particular importance due to the high mortality rate from encephalomyelitis in humans infected with this simian herpesvirus [59]. Humans become infected with this virus following a bite or scratch from a macaque monkey. Fever, lymphangitis, lymphadenopathy, gastrointestinal symptoms, and myalgia follow development of erythema, induration, and vesicles at the inoculation site. Rapid progression to the neurologic signs and symptoms of encephalomyelitis follow these symptoms [60,61]. The prognosis is very poor with B virus infection.

Pathology. Ballooning degeneration and cell fusion are seen with HSV-1, HSV-2, and VZV infections, resulting in multinucleated giant cells. The uninfected stratum corneum is elevated to form a vesicle by degeneration of epithelial cells and influx of edema fluid. Infiltration by leukocytes forms pustules. Both intranuclear and intracytoplasmic inclusions may be seen in cells infected with CMV. Intranuclear inclusions in HSV or VZV infected cells are similar to those observed in cytomegalic cells, but the CMV intranuclear inclusions are larger, surrounded by a clear halo, and resemble "owls' eyes." Viral infection of the vascular endothelium and subsequent destruction of blood vessels cause cutaneous ulcerations in CMV.

It is not certain whether EBV enters epithelial cells by interaction with a specific receptor, e.g., CD21, or by fusion of the epithelial cell with an infected lymphocyte. It is not completely understood how EBV produces a rash in infectious mononucleosis (with or without ampicillin) or in oral hairy leukoplakia.

It is not fully known how HHV-6 produces an exanthem. The presence of HHV-8 sequences has been documented in endothelial cells of Kaposi's sarcoma. Ballooning degeneration, multinucleated cells, vesicle formation, and necrosis are seen in cutaneous lesions with B virus infection.

Laboratory Findings. The most definitive method of demonstrating a herpesvirus as the probable cause of a vesicle is viral culture. In 1–2 days, both HSV-1 and HSV-2 grow readily. VZV has a much lower recovery rate than HSV and requires 7–10 days to produce a cytopathic effect. Culturable virus is much less likely to be found in pustules than in vesicles; virus can only rarely be cultured from crusts. HSV or VZV can be grown in either fibroblast or epithelial (amnion) cell cultures, but CMV only grows in fibroblast cultures. EBV or HHV-6 grows in lymphocytes; growth of HHV-8 in the laboratory has been reported recently.

Multinucleated giant cells of HSV and VZV will be revealed by scraping the base of a vesicle and subsequent staining (Tzanck smear). The Tzanck smear can differentiate HSV- or VZV-associated changes from those associated with nonviral etiologies similar to a skin biopsy or electron microscopy, but it cannot distinguish among HSV-1, HSV-2, and VZV. Differentiation of skin lesions associated with each of these three viruses can, however, be accomplished using direct fluorescent antigen staining.

Diagnosis of infection with herpesviruses is possible via serologic tests. ELISA testing is used to detect antibodies to HSV-1 or HSV-2. Western blotting provides differentiation of antibodies with high sensitivity and specificity. True primary genital herpes can be differentiated from first episode, nonprimary genital herpes by the predominance of IgM in the former and IgG in the latter presentation. A four-fold or greater increase in the antibody titer to VZV between acute and convalescent titers can retrospectively diagnose herpes zoster.

Any of the eight human herpesviruses can be detected via PCR. While this technique is the most sensitive, it is also associated with a significant incidence of false positivity if proper controls are not used.

Management. Thirteen antiviral drugs are FDA approved for therapy of herpesvirus infections (Table 1.9) [62]. Ophthalmic preparations of trifluridine and vidarabine are used

Table 1.9 FDA-approved Anti-herpesvirus Agents

Human herpes virus	Generic name	Trade name
Herpes simplex virus 1 & 2 and/or herpes zoster virus	Acyclovir	Zovirax
	Valacyclovir	Valtrex
	Famciclovir	Famvir
	Foscarnet (Acyclovir resistant HSV and VZV)	Foscavir
	Penciclovir (topical only)	Denavir
	Trifluridine (optical only)	Viroptic
	Vidarabine (optical only)	Vira A
	n-docosanol (topical only)	Abreva*
Cytomegalovirus	Ganciclovir	Cytovene, Vitrasert
	Valganciclovir	Valcyte
	Foscarnet	Foscavir
	Cidofovir	Vistide
	Fomivirsen (intravitreal only)	Vitrasene
Human herpesvirus-8 (AIDS-related Kaposi's sarcoma)	Interferon-α	Roferon-A Intron-A

*Over the counter, has antiviral activity, but not specifically approved as an antiviral drug.

for treatment of HSV- and VZV-associated keratitis and keratoconjunctivitis. Topical, oral, and intravenous formulations of acyclovir are available. Topical acyclovir has very low efficacy, but continues to be used for therapy of HSV infections. Oral, genital, and other HSV infections are treated with oral acyclovir [63]. Primary varicella and herpes zoster require a fourfold higher dose of acyclovir [64,65]. Frequent dosing and low (i.e., 15–20%) bioavailability limit the efficacy of oral acyclovir. Therefore, the intravenous preparation is favored in immunocompromised patients with HSV or VZV infections, especially with disseminated disease. Acyclovir is not only very effective in suppressing signs and symptoms of genital herpes, but was also demonstrated to reduce asymptomatic viral shedding of HSV-2 by 95% [66–68].

Two additional drugs, famciclovir and valacyclovir [69,70], were approved for treatment of herpes zoster to overcome the limitations of oral acyclovir. More convenient dosing and greater bioavailability after oral dosing are provided by famciclovir and valacyclovir than with acyclovir. These newer drugs are also approved for episodic treatment of recurrent genital herpes. Penciclovir, a metabolite of famciclovir, and n-docosonal are approved in topical formulations for the therapy of herpes labialis. Fomivirsen is an antisense compound directed against CMV and is given via intraocular injection. Fomivirsen, valganciclovir, cidofovir, foscarnet, and ganciclovir are approved for treatment of CMV infections. The latter three drugs are administered intravenously and are associated with markedly higher rates of toxicities than the three antiviral agents approved for systemic therapy of HSV and VZV infections. Ganciclovir in oral and ocular implant forms is approved for CMV prophylaxis in immunocompromised patients; valganciclovir is approved for induction and maintenance therapy of CMV retinitis; foscarnet is also approved for therapy of acyclovir-resistant HSV infections.

VZV is the only herpes virus for which a vaccine is currently available for prophylaxis (Table 1.5) [71]. Approval for this live, attenuated viral vaccine (Oka strain) came in 1995 for prevention of primary varicella (chickenpox); the vaccine produces a 95% seroconversion rate. Approval of a more concentrated form for prevention of herpes zoster (shingles) came in 2006. Studies are ongoing with recombinant glycoprotein vaccines for the prophylaxis (and possible therapy) of HSV infections [72].

Parvoviruses

The only parvovirus know to infect humans is parvovirus B19, which is the cause of erythema infectiosum (Fifth disease) (Table 1.8) and papular pruritic socks and gloves syndrome [73]. Erythema infectiosum presents most commonly in children and often occurs in epidemics in late winter and early spring [74]. This syndrome begins with nonspecific symptoms approximately 4–14 days after exposure to parvovirus B19, which is transmitted primarily by the respiratory route. Erythematous confluent, edematous plaques appear on the cheeks after about 2 days of low-grade fever, headache, and coryza. A "slapped" appearance of the cheeks is accompanied by continuation of the above-mentioned symptoms and the appearance of cough, conjunctivitis, pharyngitis, malaise, myalgias, nausea, diarrhea, and occasional arthralgias. The facial rash fades after 1–4 days concomitant with the appearance of erythematous macules and papules with a reticulated pattern on the extensor surfaces of the extremities, neck, and trunk. The rash usually lasts for 1–2 weeks, but can persist for months and can be pruritic. Since parvovirus B19 is not usually found in respiratory secretions or in the serum after the appearance of cutaneous manifestations, patients with erythema infectiosum appear to be infectious only before the appearance of the rash.

Acute arthropathy, usually without rash, is often associated with primary parvovirus B19 infection in adults. Transient aplastic crisis in patients with chronic hemolytic anemias, parvovirus-related chronic anemia in immunocompromised patients, and nonimmune fetal hydrops are other clinical presentations of parvovirus B19 infection potentially much more serious than erythema infectiosum but, uncommonly accompanied by rash.

Pathology. Although the rash appears 17–18 days following infection, viremia appears 6–14 days after a susceptible patient contracts parvovirus B19 via the respiratory route. The pathogenesis of erythema infectiosum is not understood and may relate to immune complex formation, but the systemic manifestations of parvovirus B19 infection involve viral lysis of erythroid precursor cells. There are no diagnostic histologic changes in the skin of these patients.

Laboratory Findings. In erythema infectiosum recent infection is indicated by detection of serum IgM directed to parvovirus B19 via RIA or ELISA. After 1 month, serum levels of IgM start to decline, but are still detectable for 6 months after infection. One week following infection, parvovirus B19-specific IgG can be detected and persists for years. Less readily available tests exist for detection of the virus, such as RIA, CIE, ELISA, dot blot hybridization, and PCR. Human erythroid progenitor cells can be used to culture the virus.

Management. Treatment of erythema infectiosum is aimed at relief of symptoms since no antiviral therapy exists for parvovirus B19. Development of a vaccine appears feasible because one infection with the virus produces lifelong immunity.

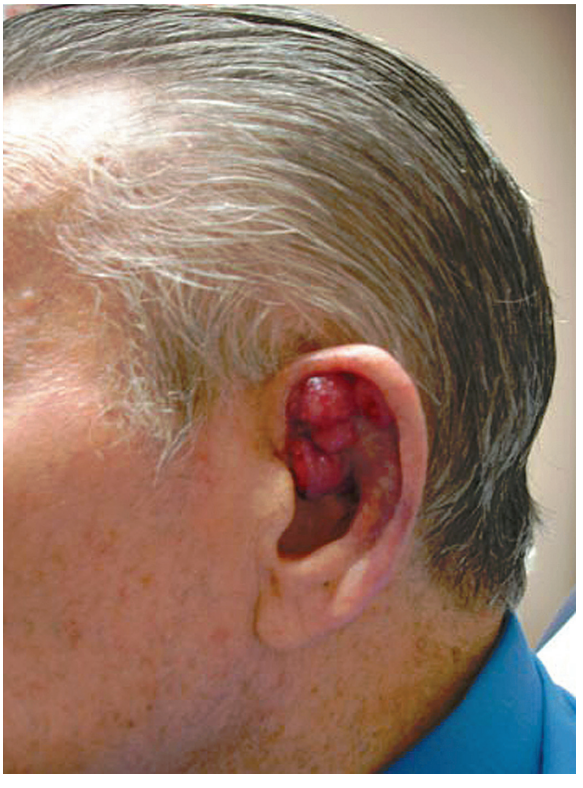


Figure 1.4 Merkel cell carcinoma, which was rapidly fatal despite aggressive therapy and was found to contain DNA from the Merkel cell polyomavirus.

Polyomaviruses

Polyomaviruses are small DNA viruses with oncogenic potential, but they usually persist in the host without causing disease. The polyomavirus that has been most closely associated with serious disease in humans is the JC virus, which can cause fatal progressive multifocal leukoencephalopathy in AIDS patients and other immunocompromised persons. In 2008, a new species, the Merkel cell polyomavirus was described and has been associated with the majority of cases of the highly aggressive Merkel cell carcinoma studied thus far (Fig. 1.4).

RNA VIRUSES

Enteroviruses

Enteroviruses, such as coxsackieviruses, are small RNA viruses belonging to a subgroup of the family Picornaviridae. The two most distinctive clinical syndromes are HFMD and herpangina, although a variety of enteroviruses, particularly coxsackieviruses, cause mucocutaneous manifestations. Most epidemic cases of HFMD are associated with coxsackievirus A16, but HFMD may be associated with coxsackieviruses A4–7, A9, A10, B2, or B5 as well as echovirus 71. Coxsackieviruses A2, A4, A5, A6, A8, or A10 cause herpangina.

Hand-Foot-Mouth Disease

Persons in their preteen to early teen years are most susceptible to HFMD [76]. This disease should be distinguished from foot-and-mouth disease (FMD) or hoof-and-mouth disease, which is a viral disease of cloven-hoofed animals. Although FMD is due to a picornavirus, the disease very rarely affects humans. HFMD is spread via oral or fecal-oral routes. A prodrome characterized by low fever, malaise, and abdominal or respiratory symptoms develops after an incubation period of 3–6 days; this prodrome precedes the mucocutaneous lesions by 12–24 hours. Most common on the hard palate, tongue, and buccal mucosa, oral lesions begin as macules that rapidly progress to vesicles and then to shallow, yellow-to-gray ulcers with an erythematous halo. Concomitant with or soon after the oral lesions, cutaneous vesicles appear and are most prevalent on the hands and feet. Cutaneous and oral lesions are usually tender or painful. In 5–10 days, both types of lesions resolve without treatment.

Herpangina

Herpangina is caused by viruses spread via routes similar to those causing HFMD. Children from 1 to 7 years of age are most commonly seen with herpangina, which begins abruptly with fever, sore throat, dysphagia, and malaise [77]. Small gray-white vesicles surrounded by erythema appear on the posterior palate, uvula, and tonsils. The vesicles usually ulcerate. Within 4–5 days, systemic symptoms usually resolve, and the ulcers heal spontaneously within 1 week.

Pathology. Viral infection of the buccal mucosa extends to regional lymph nodes in HFMD or herpangina. A viremia carries the virus to mucocutaneous sites approximately 48 hours later, resulting in intraepidermal vesicles containing neutrophils, mononuclear cells, and proteinaceous eosinophilic material. A perivascular polymorphous infiltrate composed of lymphocytes and neutrophils is observed in the edematous subvesicular dermis.

Laboratory Findings. A mild leukocytosis (i.e., 10,000 to 15,000/mm^3) may be seen in both HFMD and herpangina. The responsible virus may be recovered using tissue culture techniques or type-specific serology can identify the responsible coxsackievirus.

Management. Since no vaccine or antiviral drug is available for HFMD or herpangina, management is symptomatic.

Paramyxoviruses

Paramyxoviruses (Paramyxoviridae), such as measles, are enveloped, single-stranded RNA viruses.

Measles

Measles (rubeola) is seen primarily in winter and spring and is most prevalent in children 5–10 years of age. An incubation period of 10–11 days precedes a prodromal phase. The prodrome is characterized by high fever, cough, coryza, conjunctivitis, malaise, and Koplik's spots on the buccal mucosa, which persist for 3–4 days. Developing first behind the ears and over the forehead, an erythematous macular and papular rash then spreads to the face, neck, trunk, and extremities within 3 days. The fever, cough, and conjunctivitis are most severe when the lesions reach confluence over the face and upper back. Two to three days after the appearance of the rash, the Koplik's spots disappear. The rash fades within 5 or 6 days, sometimes with fine desquamation. Encephalitis and purpura are uncommon complications. Subacute sclerosing panencephalitis is a rare, but usually fatal, complication of measles. Persons previously given killed measles virus vaccine, who are subsequently exposed to wild-type measles or to live attenuated measles virus vaccine, can develop atypical measles [78].

Pathology. Respiratory secretions spread the measles virus throughout the prodrome until 4 days after initiation of the rash. The appearance of skin lesions concomitant with detectable serum antibody suggests that virus-antibody complexes may initiate the damage, although the rash may be partly due to viral damage to epithelial and vascular endothelial cells. Likewise, immune complexes may cause the complications of encephalitis and thrombocytopenic purpura. Hyaline necrosis of epithelial cells, formation of a serum exudate around superficial dermal vessels, proliferation of endothelial cells followed by a leukocytic infiltrate of the dermis, and lymphocytic cuffing of vessels are seen in biopsies of the measles exanthem.

Laboratory Findings. In measles, routine laboratory tests are usually unremarkable, but specific tests can include viral culture or detection of viral antigens in secretions. Measles infection is confirmed more commonly via serology using ELISA, complement fixation, neutralization, or hemagglutination inhibition tests.

Management. Treatment is supportive therapy since no approved antiviral drug exists for measles. The live attenuated measles vaccine for prevention is effective (Table 1.5). If serum immunoglobulin is administered within 6 days of exposure to the virus, passive immunity may modify or prevent measles (Table 1.6).

TOGAVIRUSES

Togaviruses (Togaviridae), such as rubella and chikungunya, are enveloped, single-stranded RNA viruses.

Rubella

Between 14 and 21 days following exposure to the virus, the prodrome of rubella (German measles, 3-day measles) develops and becomes more prominent with the increasing age of the patient. Low-grade fever, headache, conjunctivitis, cough, sore throat, and marked lymphadenopathy can be observed during the prodrome; arthritis can be seen in adults. An erythematous macular to papular rash appears first on the face and then on the neck, trunk, and extremities from 1 to 4 days after initiation of the prodrome. The rash clears with fine desquamation after 2–3 days. During spring months, rubella is most common. Intrauterine infection produces congenital malformations in 50% of infected neonates, but the sequelae of rubella are rare in children and adults. The teratogenic findings, which can affect a wide variety of organ systems, especially the heart, eye, auditory system, bone, and CNS are most severe if the infection is early during pregnancy. The characteristic cutaneous findings of the TORCH syndrome, petechiae and ecchymoses, are produced by infection of the bone marrow [79,80].

Pathology. The virus can be recovered from the pharynx from 7 days before the rash until almost 2 weeks after the rash and is spread via the respiratory route. The initial measurable antibody response appears simultaneously with the rash, suggesting that the exanthem may be due to the inflammatory effects of antibody-virus complexes rather than direct viral infection of the vascular endothelium. Only nonspecific acute and chronic inflammatory changes are seen in skin biopsies of the rubella rash.

Laboratory Findings. The peripheral blood may contain increased numbers of atypical lymphocytes and plasma cells, but they are not diagnostic. Hemagglutination inhibition, RIA, ELISA, etc., are serologic tests to detect rubella antibodies. Viral RNA can be found with PCR, or the virus may be cultured. Direct immunofluorescence may detect viral antigen.

Management. Since no antiviral drug is approved for rubella, treatment is symptomatic. A live attenuated vaccine that is administered along with the mumps and measles vaccines (i.e., MMR) is used for prevention (Table 1.5).

Chikungunya

Chikungunya is a mosquito-borne togavirus that produces epidemics in countries in the area of the Indian Ocean and is being reported more recently from various other parts of the world. The morbidity of Chikungunya is frequently severe and is similar to that of Dengue fever. The acute fever lasts 2–5 days and is followed by prolonged arthralgias affecting the joints of the extremities. The joint pain associated with Chikungunya can persist for weeks or months.

RETROVIRUSES

Retroviruses (Retroviridae) such as human immunodeficiency virus (HIV) contain a central core surrounding two identical copies of the single-stranded viral RNA genome. An envelope formed by budding from the cell membrane covers the core. HIV, like other retroviruses, contains an RNA-dependent DNA polymerase (i.e., reverse transcriptase), which allows viral RNA to be converted into a proviral DNA sequence.

Human Immunodeficiency Virus

The most important retrovirus medically, epidemiologically, and in terms of cutaneous manifestations is HTLV-III, more commonly known as human immunodeficiency virus (HIV)-l. Other retroviruses, however, such as human T-cell leukemia virus (HTLV) types I and II can have cutaneous manifestations, particularly due to the association of HTLV-I with adult T-cell lymphoma/leukemia and infective dermatitis [81].

The signs and symptoms that lead to suspicion and serologic testing for HIV in individuals at high risk are often due to the mucocutaneous manifestations of HIV infection. Progression from asymptomatic HIV infection to full-blown acquired immunodeficiency syndrome (AlDS) may be reflected in a variety of mucocutaneous manifestations [82,83]. Sexual contact with an infected person, significant exposure to infected blood or blood products (including intravenous drug abuse), or perinatal exposure are the primary routes of transmission of HIV.

The incubation period in many persons infected with HIV may be 10 or more years before the appearance of signs or symptoms. Primary HIV infection is occasionally manifested by fever and mild systemic symptoms that may be accompanied by a papulosquamous exanthem that is similar to those seen with a variety of other viral diseases. Within 2 weeks, the exanthem and symptoms generally resolve spontaneously [84].

Over 90% of patients will develop secondary mucocutaneous manifestations of their infection as the disease progresses from asymptomatic HIV infection through advanced AIDS [85,86]. Infectious, neoplastic, or noninfectious/non-neoplastic signs may be observed. Herpesviruses, poxviruses (i.e., MC), and papillomaviruses are examples of opportunistic viral infections commonly presenting clinically in HIV-positive individuals. Not only are

relatively common organisms such as *Staphylococcus aureus*, *Streptococcus*, *Pseudomonas aeruginosa*, and *Treponema pallidum* responsible for opportunistic bacterial infections, but multiple species of Mycobacterium and unique infections, such as with *Bartonella quintana* and *B. henselae* (which cause bacillary angiomatosis), also have mucocutaneous manifestations. A variety of species of tinea as well as systemic fungi, including *Candida* sp., *C. neoformans*, and *Histoplasma capsulatum* produce mycotic infections. Kaposi's sarcoma is the most common neoplasm in HIV-positive patients and is associated with HHV-8. Inflammatory diseases (e.g., psoriasis and Reiter's disease), vascular diseases, hypersensitivities to drugs, insect bites, and ultraviolet light, pruritus, xerosis, ichthyosis, and seborrheic dermatitis are all common non-neoplastic/noninfectious mucocutaneous findings.

Pathology. The virus infects CD4+ T lymphocytes by attaching to the CD4 molecule. HIV also infects monocytes and macrophages that help to spread the virus to susceptible cells in the brain, lymph node, skin, lung, and gastrointestinal tract. Disease is produced by HIV killing CD4+ cells, by syncytia formation, and by induction of certain cytokines that may play a direct role in induction of malignancy, neurologic disease, and other clinical manifestations. Several weeks are usually required after infection for detectable antibody formation to HIV; in some cases, seroconversion may follow infection by more than 1 year. The exanthem associated with primary HIV infection usually demonstrates nonspecific changes such as a superficial perivascular and perifollicular mononuclear cell infiltrate predominately composed of CD4+ cells.

Laboratory Findings. Marked leukopenia, anemia, and thrombocytopenia may be found with progression of disease, as well as an elevated erythrocyte sedimentation rate and lymphocytic cerebral spinal fluid pleocytosis. Marked declines in CD3+ cells, CD4+ cells, and a reversed CD4/CD8 cell ratio accompany disease progression. Seropositivity to the virus using ELISA with confirmation by Western blotting documents HIV infection. Isolation of the virus from the blood or demonstration of HIV p24 antigenemia demonstrates the presence of HIV.

Management. Seven synthetic nucleoside analogs, zidovudine, zalcitabine, didanosine, stavudine, lamivudine, embricitabine, and abacavir, are approved for therapy of HIV infection (Table 1.10). All seven drugs work by inhibition of HIV reverse transcription [87–91]. Four non-nucleoside analogs, nevirapine, delaviridine, etravirine, and efavirenz, are also approved. The only nucleotide analogue is tenofovir. Saquinavir was the first protease inhibitor approved for treatment of HIV. Other available protease inhibitors include ritonavir, indinavir, nelfinavir, amprenavir, darunavir, atazanavir, tipranavir, fosamprenavir, and lopinavir. There is currently one fusion inhibitor available, enfuvirtid, one integrase inhibitor, raltegravir, and one entry inhibitor, maraviroc. Combinations of antiviral drugs from different classes appear to produce greater efficacy than higher doses of individual agents, thereby reducing both adverse events and viral resistance. Such combinations usually include at least one protease inhibitor and two drugs from the other classes, although combinations of nucleoside and non-nucleoside analogs have recently been demonstrated to be effective. Together, these agents form "highly active antiretroviral therapy" (HAART). HAART has produced marked reductions in morbidity and mortality of AIDS patients since 1996. Clinical trials are ongoing with a variety of other antiretroviral drugs, as are prophylactic and therapeutic HIV vaccines. Management of HIV-positive patients also requires treatment and prophylaxis of a variety of opportunistic infections and neoplasms, in addition to drugs aimed at the responsible retrovirus [92].

Table 1.10 FDA-approved Anti-retroviral Agents

Category of drug	Generic name	Trade name
Nucleoside analogues	Zidovudine	Retrovir
	Didanosine	Videx
	Zalcitibine	Hivid
	Stavudine	Zerit
	Lamivudine	Epivir
	Emtricitabine	Emtriva
	Zidovudine + lamivudine	Combivir
	Abacavir	Ziagen
	Abacavir + Lamivudine + Zidovudine	Trizivir
	Abacavir + lamivudine	Epzicom
Non-nucleoside analogues	Nevirapine	Viramune
	Delaviridine	Rescriptor
	Efavirenz	Sustiva
	Etravirine	Intelence
Nucleotide analogue	Tenofovir	Viread
	Tenofovir + emtricitabine	Truvada
	Tenofovir + emtricitabine +efavirenz	Atripla
Protease inhibitors	Saquinavir	Invirase
	Ritonavir	Norvir
	Indinavir	Crixivan
	Nelfinavir	Viracept
	Amprenavir	Agenerase
	Tipranavir	Aptivus
	Fosamprenavir	Lexiva
	Darunavir	Prezista
	Atazanavir	Reyataz
	Lopinavir + ritonavir	Kaletra
Fusion inhibitor	Enfuvirtide	Fuzeon
Integrase inhibitor	Raltegravir	Isentress
Entry inhibitor	Maraviroc	Selzentry

Miscellaneous Viruses

Hepatitis B, hepatitis C, several hemorrhagic fever viruses and a variety of other viral diseases have occasional cutaneous manifestations, but pathogenesis and histology of the rash in these diseases are often not well understood, or the eruption may not be specific for a particular viral infection (Table 1.11). Therefore, the rash of these viral infections may be of less diagnostic or prognostic significance as in the previously discussed viral diseases. Although these viruses are covered by individual chapters in this text, viruses having no (or very rare) mucocutaneous manifestations, such as rhinoviruses, influenza, respiratory syncytial virus, rabies, etc., are not discussed further (Table 1.12).

Recognition of characteristic mucocutaneous manifestations, however, of a variety of viral diseases either directly helps to determine the etiologic agent or assists the clinician in deciding which additional diagnostic tests to order. Proper management of the patient can be initiated from the results of such tests.

An important concept in the control of viral diseases is that antiviral drugs are generally virostatic, not virocidal.

Table 1.11 FDA-approved Anti-hepatitis Agents

Hepatitis virus	Generic name	Trade name
Hepatitis B virus	Interferon-alfa-2b	Intron A
	Peginterferon alfa-2a	Pegasys
	Lamivudine	Epivir-HBV
	Entecavir	Baraclude
	Adefovir	Hepsera
	Tenofovir	Viread
	Telbivudine	Tyzeka
Hepatitis C virus	Interferon-α	Roferon-A, Intron A, Infergen
	Interferon-α + ribavirin	Rebetron
	Peginterferon Alfa-2b + ribavirin	PegIntron + Rebetol
	Peginterferon alfa-2a + ribavirin	Pegasys + Copegus

Therefore, prevention of viral infections takes on an even greater level of importance. Such control includes good public health measures, such as sanitation, hand washing (and the use of disposable examination gloves), safe sex (abstinence, condoms), control of mosquitoes (and other vectors), testing of blood products, and single use of needles. Otherwise, the single most effective medical intervention is the use of vaccines. The prototype of a successful vaccine campaign was the eradication of smallpox. Since widespread vaccination of the general public stopped over 20 years ago, the majority of the world's population has no immunity to smallpox. Therefore, smallpox is considered to be a leading pathogen that could be used for bioterrorism.

Generally, the FDA-approved vaccines are several orders of magnitude safer, in terms of morbidity and mortality, than the diseases that they are designed to prevent. One vaccine, however, was removed from the market due to safety issues. Rotashield® was a live, oral tetravalent, rotavirus vaccine that was associated with several cases of intussusception and is considered to be causal [93]. It has been replaced by two new rotavirus vaccines, Rotarix and RotaTeq, which are not associated with increased rates of intussusception. Most associations between vaccines and adverse events are not, however, demonstrated to be causal. For example, the measles mumps rubella (MMR) vaccine was reported very recently not to have a causal relationship to autism [94,95]. Likewise, a causal relationship between the hepatitis B vaccine and a variety of autoimmune diseases has been disproven. This vaccine does not increase the risk of multiple sclerosis nor does it cause a relapse of pre-existing multiple sclerosis [96,97]. Nevertheless, suspected relationships between vaccines and adverse events need to be reported to the "Vaccine Adverse Event Reporting System" (1-800-822-7967) so that the excellent safety record of vaccines can be maintained.

Table 1.12 FDA-approved Anti-influenza Agents™

Generic name	Trade name	Indication
Amantadine	Symmetrel	Influenza A
Ramantadine	Flumadine	Influenza A
Oseltamivir	Tamilflu	Influenza A and B
Zanamivir	Relenza	Influenza A and B

It is anticipated that the future will bring safe and effective vaccines for a variety of viral diseases, e.g., HIV, hepatitis C, HSV, and new strains of influenza. Although no vaccine is available for the therapy of a viral disease, our concept of vaccines is now being expanded by ongoing clinical trials of therapeutic vaccines, e.g., for HIV, HSV, and HPV.

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2 Cutaneous Resistance to Viral Infections

Melissa C Morgan, Rashid M Rashid, and Stephen K Tyring

Introduction

The skin permits primary immune sensitization, retains immunologic memory, and houses immunocytes, and can be preferentially affected by T-cell malignancies. Based upon this information, Streilein proposed a specific relationship between the immune system and the integument, much like the gut-associated lymphoid tissue [1]. He then proposed the concept of skin-associated lymphoid tissue (SALT) [2]. SALT is composed of: (1) keratinocytes, which can phagocytize, release many cytokines, and even express major histocompatability complex (MHC) class II antigens upon incubation with interferon-γ (IFN-γ); (2) epidermal Langerhans cells (LC), dendritic cells that have surface expression of MHC class II, CD1, CD3, and CD4 molecules, and are the predominant scavenger antigen-presenting cells of the epidermis; (3) skin tropic T cells, which in the epidermis include mainly "inactive" memory T cells of predominantly CD8+ phenotype, although CD4+ and CD4−, CD8−, and γδ+ T cells are also present; and (4) skin endothelial cells, which direct cellular traffic in and out of the skin. The epidermis contains the basic elements needed for an immune response (T cells, antigen-presenting cells, and cytokines). This, in conjunction with its anatomic structure, serves as a primary line of defense against infections. Therefore, we review the components of SALT, and their interactions with viruses having cutaneous manifestations.

Langerhans Cells

LCs are the professional antigen-presenting cells of the epidermis. LCs capture exogenous antigens and process them into peptides for presentation to CD4+ T cells in the context of MHC class II. So far, two predominant methods of antigen capture have been described in LCs; one is micropinocytosis and the other involves a mannose receptor-mediated mechanism. Additionally, when infected with a virus such as human immunodeficiency virus (HIV), LCs present viral peptides to CD8+ T cells in the context of a different MHC class (class I).

Skin biopsies from 7 of 40 HIV-positive individuals reacted with anti-HIV-1 core protein in an indirect immunofluorescence assay [3]. The only cells infected with HIV that could be detected were LCs, although Heng et al. have shown that keratinocytes, which do not express CD4, can be co-infected with HSV-1 and HIV in vivo [4]. Berger et al. demonstrated that LCs could be infected with HIV in vitro, and that LCs from HIV-positive individuals could infect mononuclear phagocytes from HIV-negative individuals [5]. Cimarelli et al. quantified the proviral DNA in LCs and found that this value correlated with the frequency of peripheral blood CD4+ T cells infected with HIV in acquired immune deficiency syndrome (AIDS) patients [6]. Two important questions arise: how are the LCs infected? How do they contribute to the pathology associated with AIDS?

HIV, the causative agent of AIDS, is a retrovirus that can incorporate into cellular DNA through reverse transcriptase. Infection leads to a progressive weakening of cell-mediated immune function and a progressive decline in the numbers of peripheral blood CD4+ cells. The effect on the humoral immune system is the induction of hypergammaglobulinemia, which enables diagnosis but is not sufficient to eliminate HIV infection. HIV preferentially infects HIV-specific memory CD4+ T cells as evidenced by the fact that these cells contain more viral DNA than other memory CD4+ cells [7]. Additionally, antigen-specific T cells responding to antigens presented by dendritic cells are more likely to be infected with HIV than non-responding T cells [8].

LCs normally reside in the epidermis. Upon activation, these cells migrate to the draining lymph nodes and come in contact with T cells. By using the HIV animal model of simian immunodeficiency virus (SIV) and rhesus macaques, it was shown that dendritic cells of the lamina propria are the first to be infected with intravaginal inoculation of virus [9]. Four rhesus macaques were inoculated intravaginally and then sacrificed 2, 5, 7, and 9 days later. The animal sacrificed at 2 days post-inoculation showed productive infection only in the dendritic cells of the lamina propria. Conversely, no infection could be detected by polymerase chain reaction (PCR) in the epithelial layer, including in LCs. Interestingly, SIV-infected cells in the lamina propria were found only immediately beneath the single columnar epithelium of the endocervix. Paradoxically, other investigators have shown that up to 40% of infected cells of the vaginal tract in rhesus macaques with chronic infection of SIV are intraepithelial LCs [10]. Miller and Hue were the first to provide in vivo evidence that LCs of the genital tract are infected with SIV. These same investigators claim that the dendritic cells of the epithelial layer are the first to be infected with SIV during vaginal inoculation [10]. A later study, using an ex vivo human organ culture system, definitively showed that HIV-1 simultaneously penetrates both intraepithelial vaginal LCs and CD4+ T cells upon contact in situ [11]. HIV enters LCs mainly via endocytosis of intact virions, while CD4+ cells are infected directly via CD4 and CCR5 receptor-mediated fusion. This study further demonstrated that the majority of intraepithelial CD4+ cells express CCR5 and that LCs commonly express CD4 and CCR5 (Fig. 2.1). These findings explain the rapidity of initial HIV infection in these cell types.

Kawamura et al. found that R5 HIV infection of LCs is dependent upon the CCR5 co-receptor and that CCR5 receptor polymorphisms account for varying levels of genetic susceptibility to HIV. Specifically, individuals expressing the mutant CCR5 allele, *ORFΔ32*, in both homozygous and heterozygous forms were less susceptible to HIV infection of LCs compared to individuals who lacked the mutant allele [12]. In a later study, they found that HIV-infected LCs transmit R5 HIV to T cells in a process that is mediated by CD4 and CCR5 receptors (Fig. 2.2). Further, LCs are responsible for more than 95% of HIV dissemination to subepithelial tissues [13]. Fahrbach et al. found that activated LCs

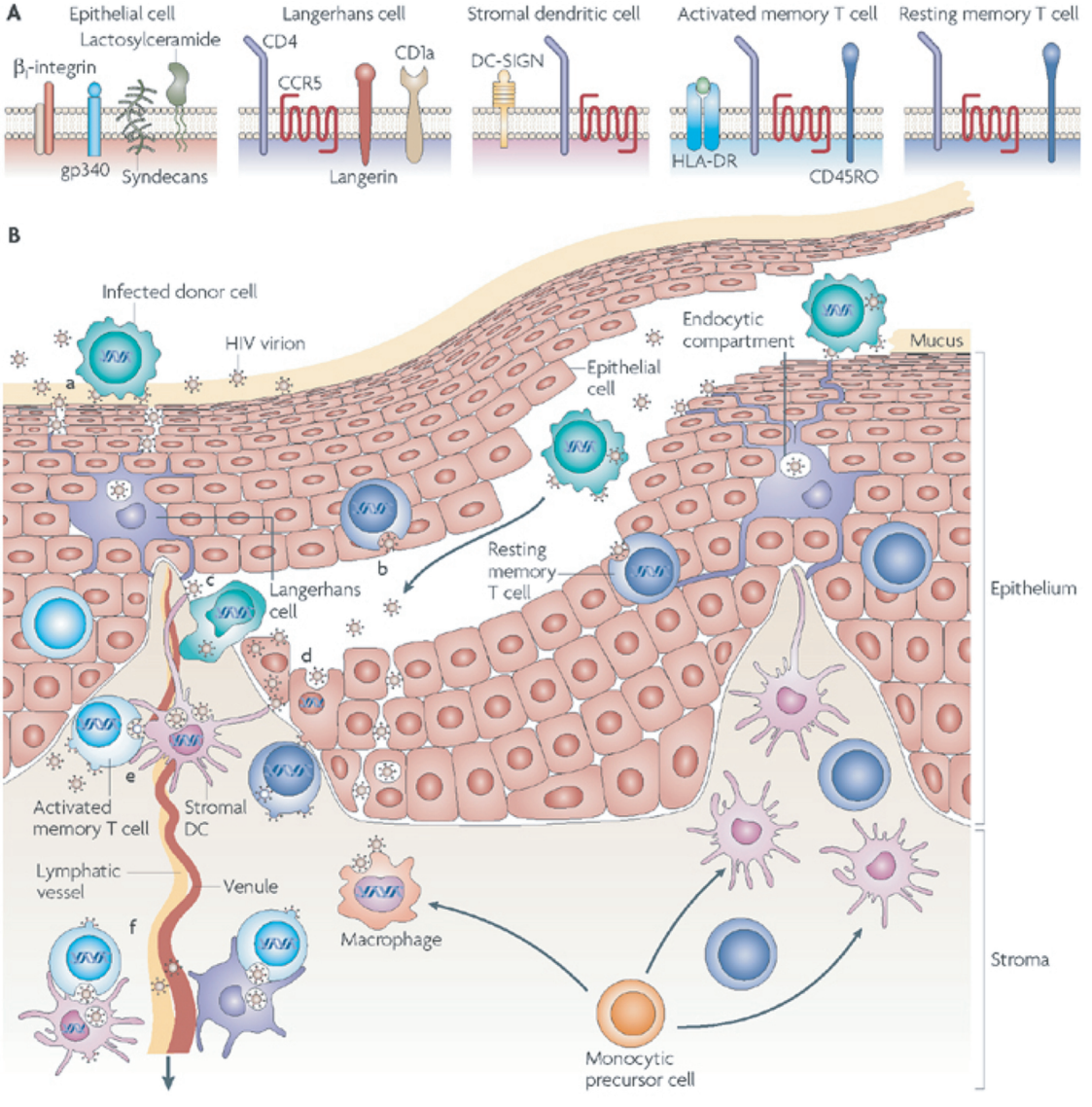

Figure 2.1 Pathways of HIV invasion in the mucosa of the vagina and ectocervix: characteristic phenotypic cell receptors and receptors relevant for HIV binding and infection are shown in A (top). The possible pathways of HIV penetration are summarized in **B**. (a) Free HIV virions or HIV-infected donor cells are trapped in mucus, resulting in penetration of the free virions into gaps between epithelial cells or attachment of HIV-infected donor cells to the luminal surface of the mucosa and secretion of virions on contact. The virions are then captured and internalized into endocytic compartments by Langerhans cells that reside within the epithelium. (b) HIV can also fuse with the surface of intraepithelial CD4+ T cells, followed by productive infection of these cells. (c) Infected donor cells or free virions can immigrate along physical abrasions of the epithelium into the mucosal stroma. There, they are taken up by lymphatic or venous microvessels and transported to local lymph nodes or into the blood circulation, respectively, or they make contact with stromal DCs, T cells, and macrophages. (d) Virions can transcytose through epithelial cells near or within the basal layer of the squamous epithelium, productively infect basal epithelial cells, be internalized into endocytic compartments, or penetrate between epithelial cells. (e) Once within the stroma, virions can productively infect stromal DCs or be internalized into the endocytic compartments of DCs and pass from the stromal DCs to CD4+ T cells across an infectious synapse where massive productive infection of CD4+ T cells ensues. In addition, virions can productively infect resting mucosal CD4+ memory T cells in the stroma and possibly stromal macrophages. (f) Productively infected CD4+ T cells and stromal DCs, and stromal DCs or intraepithelial LCs harboring virions in endocytic compartments, can emigrate into the submucosa and the draining lymphatic and venous microvessels. CCR5, CC-chemokine receptor 5; DC-SIGN, dendritic cell-specific ICAM-3-grabbing non-integrin. (Reprinted from F Hladik, MJ McElrath. Setting the stage: host invasion by HIV. Nat Rev Immunol 8: 450, 2008.)

are capable of transinfecting other target cells without becoming infected themselves. Activated LCs enhance transinfection at a rate 35 times higher than inactivated LCs. The authors suggest that transinfection is mediated by internalization of HIV virions into a trypsin-resistant compartment where they maintain infectivity and are later transmitted to target cells [14]. Blauvelt et al. propose that productive infection of dendritic cells by HIV-1 and the cell's ability to capture virus are mediated through separate pathways [15]. While productive infection is dependent on the CD4 ligand and co-receptor stimulation, HIV capture and transmission can take place independent of these factors. Epithelial LCs capture virus and deliver them to the draining lymph nodes. During activation by T cells, in the cytokine-rich lymph nodes, LCs may subsequently become productively infected. Recirculation of these LCs to the epithelial layer may explain the 40% composition of infected cells in the vagina [10].

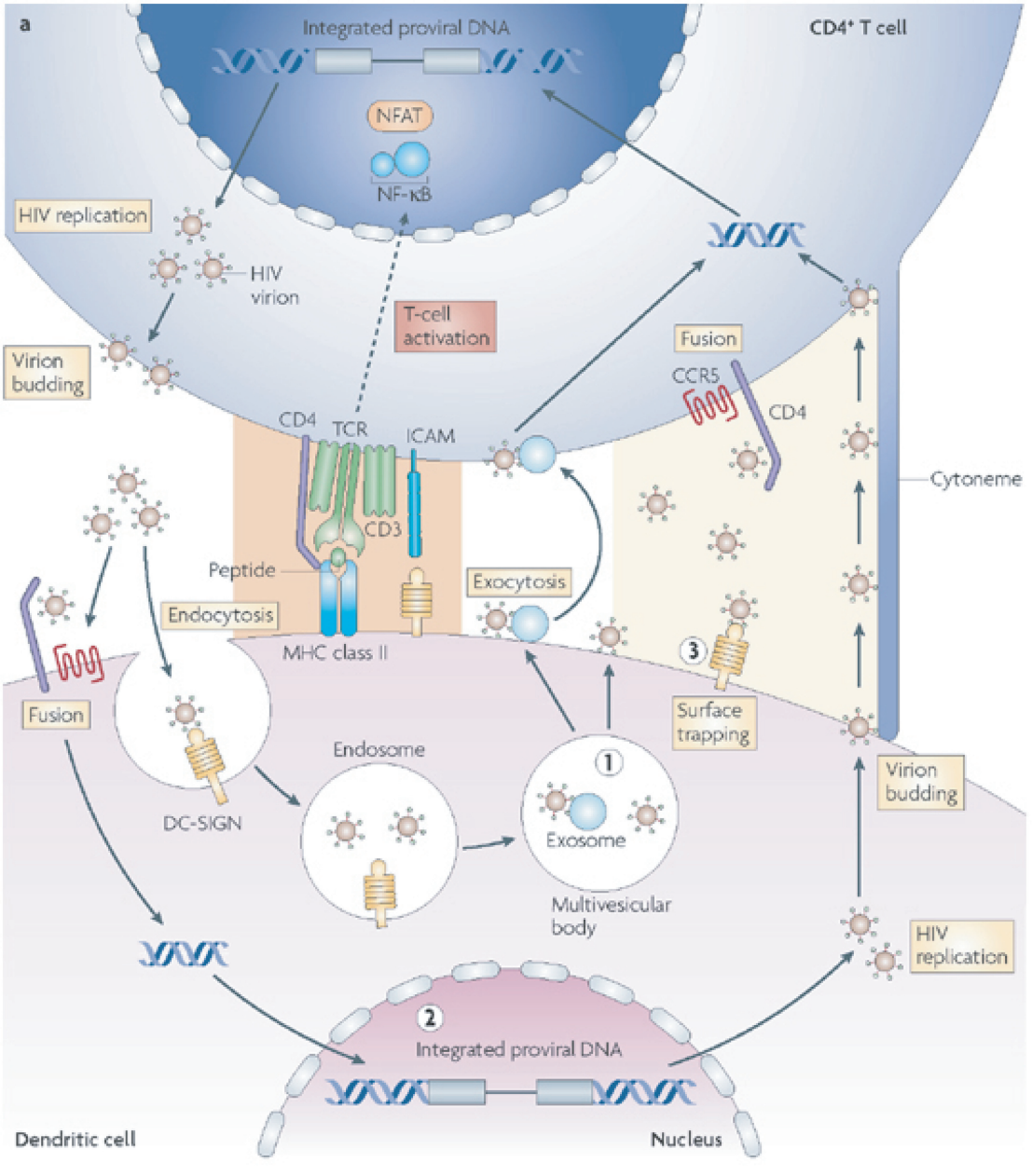

Figure 2.2 Pathways of HIV-1 passage between dendritic cells and CD4+ T cells: DCs can store HIV-1 in three forms for eventual infection of CD4+ T cells. (1) HIV can be stored as endocytosed intact virions in multivesicular bodies, following endocytic entry via C-type lectins such as DC-specific ICAM-3-grabbing non-integrin (DC-SIGN; also known as CD209). (2) HIV-1 can also be stored as an integrated provirus following entry by CD4− and co-receptor-mediated fusion, which leads to productive infection of DCs. (3) HIV can be stored as surface-bound intact virions by C-type lectins, such as DC-SIGN. Passage of HIV-1 from DCs to CD4+ T cells occurs most effectively across an infectious synapse (yellow shading), formed by a concentration of HIV-1 virions on the DC side and HIV receptors such as CD4 and CC-chemokine receptor 5 (CCR5) on the T-cell side. HIV is released into the infectious synapse either by exocytosis of stored virions from multivesicular bodies or by budding of newly formed virions following active viral replication. Virions trapped to surface receptors such as the C-type lectin DC-SIGN can also accumulate in the infectious synapse. Migration of HIV toward the T cell may be further enhanced by "surfing" of virions along the outer surface of filopodia or cytonemes that are extended from the T cell toward the DC. Coupling of virions to exosomes as they are being released from multivesicular bodies also increases their infectivity. Exosome-associated virions are likely to be transmitted to CD4+ T cells through membrane binding and fusion, either within the infectious synapse or over longer distances. In parallel with transmission of virus from the DC to the CD4+ T cell, the DC also presents antigenic peptides through MHC class II molecules to the T-cell receptor (TCR). During peptide recognition, additional receptor–ligand pairs that are important for T-cell stimulation accumulate in this region and form an immunological synapse (orange shading). Signals delivered through the immunological synapse lead to T-cell activation, which ultimately causes transcription factors such as nuclear factor-κB (NF-κB) and nuclear factor of activated T cells to translocate into the nucleus of the T cell. There, they bind to the enhancer region of the viral long terminal repeat and activate viral gene transcription, driving HIV-1 replication. (Reprinted from F Hladik, MJ McElrath. Setting the stage: host invasion by HIV. Nat Rev Immunol 8: 453, 2008.)

Consistent with this viral transfer model is the recent discovery of a new lectin, designated DC-SIGN. DC-SIGN binds ICAM-3 and HIV and maintains it on the cell surface for days, in a native conformation conducive to infection. However, LCs are not known to express DC-SIGN, but once in the dermis, dendritic cells express this lectin. Since epidermal, i.e., DC-SIGN-negative dendritic cells

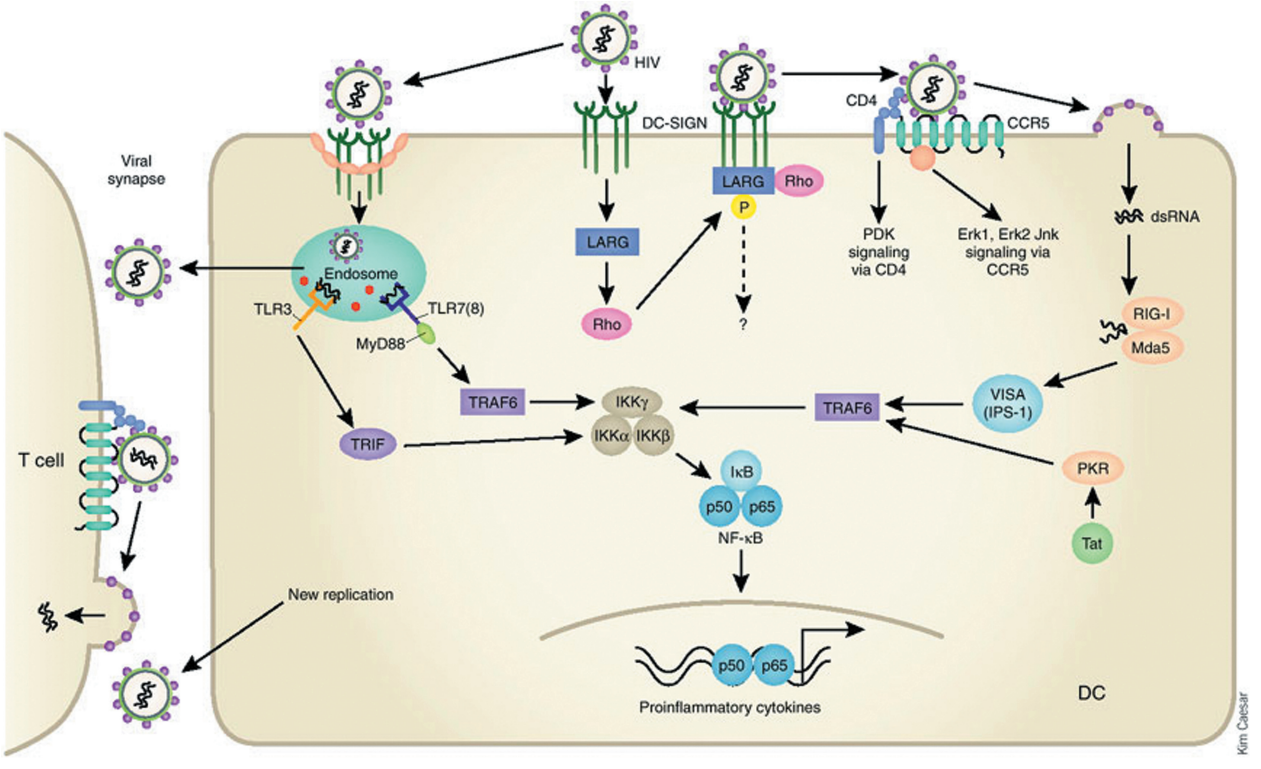

Figure 2.3 Binding of HIV-1 to DC-SIGN: binding of virus to DC-SIGN activates NF-κB through TLR3 and TLR7 (left) and/or leads to the phosphorylation of LARG and activation of Rho (middle). The LARG-Rho complex then becomes associated with DC-SIGN at the cell surface. Through an as yet unidentified mechanism ("?"), this inhibits the maturation of DCs and stimulates their ability to form synapses with T cells after HIV-1 infection. After binding to DC-SIGN, HIV can be transferred laterally ("*cis* transfer") to immature DC-expressed CD4 and CCR5 (top right); this is followed by fusion of the viral envelope (right) with the plasma membrane, delivery of double-stranded RNA (dsRNA) to RIG-1 or Mda5, and activation of NF-κB through VISA-TRAF6 (middle bottom). Transfer of HIV from DCs to T cells ("*trans* transfer") through the viral synapse (top left) occurs in two phases: first, from the DC endosome, and second, after new HIV replication (bottom left). IKK, inhibitor of NF-κB (IκB) kinase; p50 and p65, subunits of NF-κB. (Reprinted from AL Cunningham, AN Harmon, H Donaghy. DC-SIGN 'AIDS' HIV immune evasion and infection. Nat Immunol 8 (6): 557, 2007.)

are more selective for M-tropic HIV-1, the LCs likely transmit M-tropic HIV to the dermal dendritic cells, which become DC-SIGN positive to facilitate HIV transmission [16,17]. When HIV binds DC-SIGN, a virion may be endocytosed and destroyed within the dendritic cell, replicate within the dendritic cell, or be transferred from the dendritic cell to T cells (Fig. 2.3) [18].

It has been proposed that Langerin, a C-type lectin expressed on LCs, may have a function analogous to DC-SIGN on dendritic cells. However, de Witte et al. found that Langerin actually prevents HIV-1 infection of LCs by internalizing HIV virions into Birbeck granules and degrading these virions (Fig. 2.4) [19]. These results were confirmed by Kawamura et al.; however, they found that LCs may be infected when exposed to high concentrations of HIV. Thus, they suggest that Langerin may be saturated at high viral concentrations, rendering it unable to prevent HIV infection [13].

Alternatively, seeding of LCs may occur by direct inoculation and productive infection. This is particularly plausible in co-infection scenarios where, for example, a herpetic lesion has compromised the cornified layer of the epidermis. The lesions attract and activate CD4+ cells. This leaves LCs and other cells in the epidermis and possibly dermis vulnerable to primary infection. This idea is supported by the finding, in the rhesus macaques model, that the only DC-SIGN-positive cells of the lamina propria that were infected were those directly beneath the single columnar epithelium [9]. This epithelium barrier is an easier barrier to penetrate than that of squamous epithelium. Additionally, the epithelium of the vagina is not as tight and impenetrable as the skin. It is moist and fluid is continually passing through the intercellular spaces. These epithelial cells are connected by discontinuous patches of desmosomes, the weakest form of intercellular junction [9].

Another question that arises is the role of HIV on the status and function of LCs. A significant reduction of epidermal LCs in HIV-positive individuals has been demonstrated [20]. HIV infection can result in a milieu where levels of various cytokines, especially interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are chronically elevated (Fig. 2.5) [21]. These cytokines are essential and powerful stimulators of LC migration out of the epidermis [22,23]. Cytokine stimulus may partly explain the reduction of epidermal LCs in HIV infection. LCs originate from CD34+ marrow-derived cells that can differentiate along two primary pathways. One pathway leads to the formation of a group of cells most known for their expression of CD1, and they go on to become the LC of the epidermis [24]. The alternative pathway leads to the dendritic cells of the dermis, noted for expression of CD14. While the CD1+ cells of the epidermis promote cell-mediated immunity (CMI), the CD14+ cells of the dermis tend to initiate a humoral response [24]. When the hypergammaglobulinemia in HIV and deficiency of CMI are considered, speculation is that

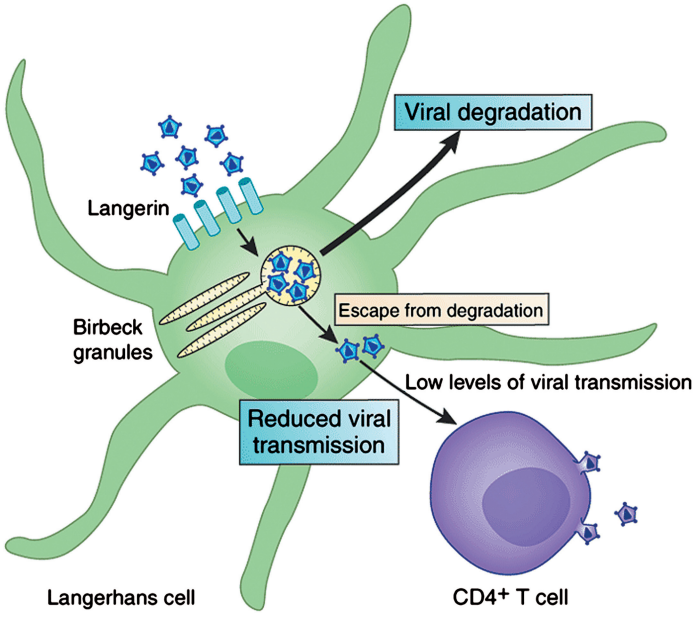

Figure 2.4 HIV-1 clearance by Langerhans cells: Langerhans cells in the mucosal epithelium are probably the first cells targeted by the virus during sexual transmission. Langerhans cells selectively express a C-type lectin, Langerin, which binds HIV-1 virions and drives them to Birbeck granules. Most of the captured virions are then rapidly degraded. Langerhans cells are thus responsible for virus clearance. However, a fraction of the incoming virions may escape degradation, in which case they will replicate at low levels in Langerhans cells and be transmitted to CD4+ T cells. (Reprinted from O Schwartz. Langerhans cells lap up HIV-1. Nat Med 13 (3): 246, 2007.)

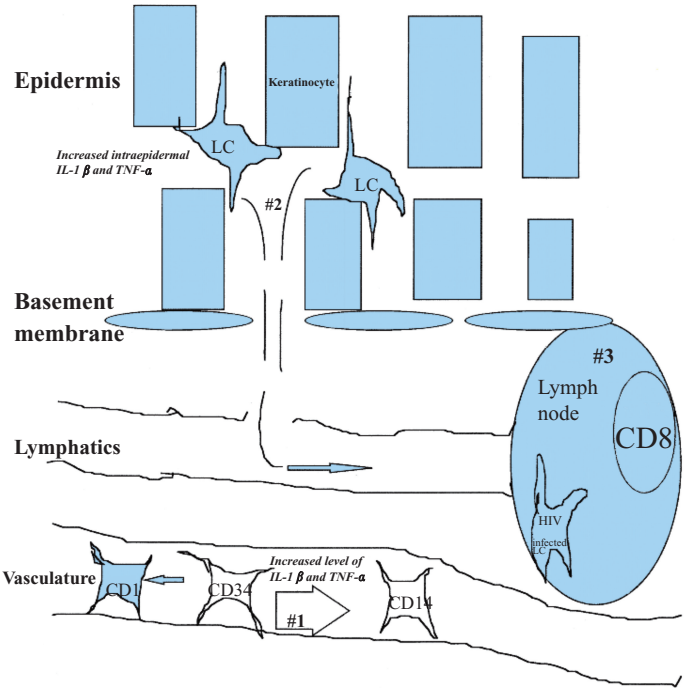

Figure 2.5 Intradermal depletion of Langerhans cells in HIV infection by three separate mechanisms: (1) IL-1β and TNF-α influence CD34 Langerhans cells precursors to differentiate towards CD14 dermal dendritic cells rather than CD1 LC; (2) Langerhans cells migrate toward the draining lymph nodes; (3) cytotoxic lymphocytes within lymph nodes destroy HIV-infected Langerhans cells. (Reprinted from AL Cunningham, Z Mikloska. The holy grail: immune control of human herpes simplex virus infection and disease. Herpes 8 (Suppl 1): 7A, 2001.)

direct or indirect cytokine manipulation by HIV results in a preference for the CD14+ pathway. This might be a second factor in the depletion of epidermal LCs. Lastly, HIV infection has definite cytopathic effects on LCs. After migrating to lymph nodes and activating T cells, dendritic cells do not leave the lymph nodes in the efferent lymphatics. In vitro studies have shown that HIV-infected dendritic cells could serve as targets of cytotoxic lymphocytes (CTL) [25]. In one study, as many as 50% of the dendritic cells were lysed after 3 days of HIV exposure, which was then followed by exposure to activated CTL.

Cytokines strongly influence HIV-infected LCs. Leonard et al. created a transgenic mouse with the HIV long terminal repeat (LTR), which contains all known HIV transcriptional response elements, linked to a reporter gene [26]. LCs from the mouse skin had a higher reporter gene activity when compared to other cells of the monocyte/macrophage lineage. This indicates that HIV provirus is easily induced in LCs. The transgenic mouse macrophages treated with a variety of cytokines (colony-stimulating factor-1, granulocyte-monocyte colony-stimulating factor (GM-CSF), IL-1α, and IL-2) had much higher reporter gene activity than macrophages incubated in the absence of these cytokines. These results indicate that the above cytokines are involved in modulating SALT, and it would be important to elucidate their role in human skin from HIV-positive patients.

In healthy skin, IL-1 is constitutively made by keratinocytes, whereas LCs make IL-1 upon activation and depend upon it for their proper maturation [27]. IL-1 upregulates IL-2 and IL-2 receptor production by T cells, a process necessary for an antigen-specific immune response. Stage of disease, by CDC classification, has been correlated with epidermal LC numbers. Subsequently, Dreno et al. have shown a relationship between intraepidermal levels of IL-1 in normal skin of HIV-positive patients and the stage of their disease [28]. All stage II patients had high levels of intraepidermal IL-1. Significant decreases in IL-1 were seen in stage III patients, with even lower or in some cases undetectable levels in stage IV^c and stage IV^d patients. It is likely that the paucity of intraepidermal LCs in these later stages is directly responsible for the lower levels of IL-1. These lower levels of IL-1 leave the epidermis devoid of T cells, vulnerable to infections, at risk for development of neoplasms, and anergic to recall antigens.

In addition to decreased epidermal LCs, symptom-free HIV-positive individuals have reduced intraepidermal CD4+ cell counts. After IL-2 injection, however, there is a local accumulation of T cells, monocytes, and LCs [29]. Although epidermal infiltration of CD4+ cells is normally reduced in HIV-positive patients, IL-2 induces a CD4+ infiltrative response equivalent to that seen in HIV-negative individuals and results in enhanced recall response to antigens [4]. Larsson et al. showed that dendritic cells can cross-present HIV antigens from both live and apoptotic monocytes carrying infectious and non-infectious HIV-1 in order to activate HIV-specific CD4+ and CD8+ T cells [30]. Maranon et al. found that dendritic cells can cross-present very small amounts of HIV proteins from both live and apoptotic HIV-infected CD4+ T cells. Therefore, inoculation of IL-2, which is usually made by T cells in response to IL-1, could be utilized to induce HIV expression and eradicate latently infected HIV reservoirs [31].

A great majority of T cells in the human epidermis have $\alpha\beta$ T-cell receptors. Some of these T cells lack the co-receptors CD4

and CD16 and their function in the epidermis is unknown. In humans, epidermal γδ+ cells are not dendritic and are involved in many diseases. CD1, which is expressed on LCs, can act as an antigen-presenting molecule for γδ+ T cells [32]. γδ+ cells, through non-MHC-restricted cytolytic activity, contribute greatly to immune surveillance against malignancies and viral infections [33]. In HIV infection, local γδ subtype ratios of bronchial-associated lymphoid tissue (BALT) are altered relative to HIV-negative individuals [34]. Hennier et al. observed higher γδ+ cells in the blood of relatively healthy HIV-positive patients than in symptomatic HIV-positive patients [35]. HIV-positive patients with oral candidiasis had even lower blood γδ T-cell counts. Numerous infectious, neoplastic and idiopathic cutaneous manifestations in HIV patients, along with the known effects of HIV on γδ+ cells in other lymphoid tissues, would suggest that γδ+ cells are functionally affected in the skin of HIV-positive patients. Unfortunately, it is not known whether γδ counts increase or decrease in the epidermis of HIV-positive individuals. Indirect immunofluorescence assays of punch biopsies of skin using pan-γδ antibodies should be a simple and direct method of answering this question.

A decrease in intraepidermal LCs is a common phenomenon of viral infections; however, some viruses have adapted other mechanisms of influencing LCs (Table 2.1). Mature dendritic cells release the cytokines necessary for T-cell activation and to ward off HSV infections [36,37]. Salio et al. showed that infected dendritic cells are unable to upregulate co-stimulatory molecules, do not produce cytokines and do not acquire responsiveness to those chemokines required for migration to secondary lymphoid organs [38]. Kruse et al. demonstrated that HSV infection of dendritic cells leads to impaired T-cell stimulatory capacity and degradation of the cell surface marker, CD83, which is upregulated during maturation of dendritic cells [39]. HSV infection of dendritic cells leads to their apoptosis. Bosnjak et al. showed that apoptotic HSV-infected dendritic cells are phagocytosed by uninfected dendritic cells, which then stimulate HSV-specific CD8+ T cells [40]. A recombinant replication defective HSV-1 encoding a green fluorescent protein is used to compare infected and uninfected dendritic cells. Normally, dendritic cells are the primary producers of IFN-α. It may be that the dysregulation of dendritic cells by HSV, as described above, allows HSV to evade the immune system [41].

Keratinocytes

Keratinocytes are squamous epithelia that take part in SALT by production of cytokines, presentation of endogenous viral antigens in the context of MHC class I to CD8+ T cells, and expression of MHC class II when stimulated with IFN-γ. Human papilloma viruses (HPV) are DNA viruses that can directly infect keratinocytes (Fig. 2.6). HPV gains entry into the epidermis through a break in skin and remains in the basal layer. It replicates just below the granular layer and gives rise to a slowly growing lesion. There are over 100 different recognized HPV genotypes [42]. HPV are categorized by regional tropism and potential for malignant transformation. For example, HPV-6 and -11 are tropic for the genital skin and mucous membranes, giving rise to condyloma acuminata, which has a low probability of undergoing malignant transformation. Epidermodysplasia verruciformis (EV) is characterized by persistent disseminated wart-like skin lesions associated with a variety of unique HPV types, e.g., HPV-5 and -8. One-third of patients with EV later experience malignant transformation of the HPV-infected lesions.

Recently, the active role of SALT in HPV infections has been further elucidated. Generally, regressing viral lesions are accompanied by CD4+ and CD8+ cellular infiltrates, an increase in epidermal LCs, an increase in dermal dendritic cells, and the appearance of human leukocyte antigen (HLA)-DR+ keratinocytes in the dermis. HPV lesions have a reduced number of epidermal LCs, which Drijkoningen et al. propose is likely due to direct cytotoxic effects of the virus [43]. Furthermore, lesions positive for HPV viral antigens are more likely to have reduced HLA-DR+ cell counts in the epidermis [44]. Keratinocytes are known to express

Table 2.1 Alterations in Langerhans Cells Following Viral Interaction

Virally-induced changes in Langerhans cell structure and function
Increased IL-1*β* in early stages of HIV infection
Increased TNF-*α*
Increased Langerhans cells migration out of epidermis
Decreased IL-1 in late stages of HIV infection
Decreased IL-2 in late stages of HIV infection
Decreased antigen presentation ability
Decreased T cell stimulatory capacity
Increased apoptosis of Langerhans cells

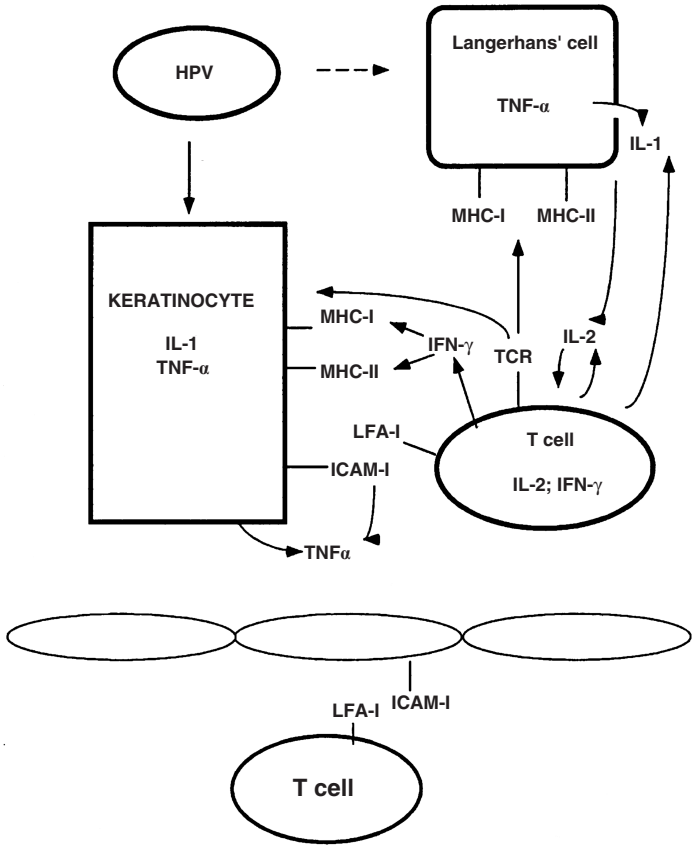

Figure 2.6 Necessary immune mechanisms for effective HPV clearance, including MHC-II presentation of HPV antigens to CD4 cells by Langerhans cells with co-stimulation by B7 and stabilization by ICAM-1. MHC-I and -II, IL-1, TNF-α, IL-2, ICAM-1, IFN-γ, and co-stimulatory molecules are commonly deficient in chronic HPV infections.

HLA-DR in HPV infections, but not HLA-DQ; therefore, staining for HLA-DR in the lesion reveals its expression on keratinocytes and not LCs. Viac et al. observed HLA-DR+ keratinocytes only in condyloma and laryngeal papillomas and not in palmar and plantar verruca [45]. HLA-DR expression directly correlated with the intraepithelial upregulation of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1) [45]. ICAM-1 is expressed on keratinocytes in condyloma and not in verruca plana. LFA-1, the natural ligand for ICAM-1, is expressed on lymphocytes and directs lymphocytes to the epidermis. Normally, ICAM-1 is expressed at low levels on dermal endothelial cells, not the epidermis. Upregulation of ICAM-1 and other adhesion molecules leads to lymphocytic infiltration. This, along with upregulation of HLA-DR in the epidermis, could facilitate antigen presentation to infiltrating CD4+ T cells and lead to clearance of infection [46–47].

We have addressed the role of HPV pathology in skin by identifying HPV gene products and immunological and cellular responses in patients. We probed for TNF-α and TGF-β1 levels in HPV-6 and -11 induced condylomas and found a dramatic reduction in their levels when compared to normal skin [48]. Majewski et al. found increased expression of TNF-α and TGF-β1 in EV lesions [49]. The disparity of TNF-α and TGF-β1 levels between EV and condyloma might indicate part of the underlying defect in EV patients. There may be a lack of proper T-cell repertoire [50], which is necessary to mount a regulatory or effector function, a cytokine receptor defect [51], or active neutralization of the cytokines by HPV products [52]. Ramoz et al. discovered that EV is associated with nonsense mutations in the genes EVER1 and EVER2 [53]. It was then determined that the gene products of EVER1 and EVER2 are transmembrane proteins in the endoplasmic reticulum (ER), which may act as ion transporters or channels or as modulators of these structures [54]. Orth later hypothesized that EVER proteins act as constitutively expressed restriction factors in keratinocytes and that EV is caused by a primary deficiency in HPV-specific intrinsic immunity [55]. This dysregulation at the level of the ER is also believed to be the cause of the pathologic finding of koilocytes (Koilo being Greek for hollow), which may be due to an enlarged ER. It is also interesting to note that, despite the presence of a mutation, treatment modalities around these defects are possible. For example, Berthelot et al. were able to successfully treat a patient with EV and an EVER2 mutation with topical imiquimod [56]. Imiquimod induces secretion of pro-inflammatory cytokines, predominantly IFN-α, TNF-α, and IL-12, thereby favoring a Th1 immune response, which is useful in the treatment of viral infections [57].

The viral oncoprotein/cellular protein interactions are well established in "high-risk" HPV types, but very little is known concerning lesions caused by "low-risk" HPV types. mRNAs of the early E2, E5, E6, and E7, as well as the late L1, can be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in "low-risk" HPV containing condylomas [48]. Especially important is the high abundance of E7 and E6 messages, since the former two can inhibit pRb and p53, respectively, two well-known tumor suppressor genes. Also important is E2, which regulates expression of HPV genes. The L1 gene encodes the major capsid protein and plays a critical antigenic role in cellular immunity. Viral oncoproteins, such as "high-risk" E6 and E7 interact with cellular regulatory proteins (e.g., p53, pRb, E2F) by displacing them in specific cellular pathways. "Low-risk" E6 and E7 proteins are probably not capable of binding cellular proteins, or at least bind with lower affinity, yet they are still able to transregulate certain host genes, as do their "high-risk" counterparts [58,59].

In condyloma lesions, there are decreased levels of growth-inhibitory genes (TGF-β1 and p53). Also, increased mRNA levels of hyperphosphorylated (inactive) retinoblastoma tumor suppressor gene product (pRB), reduced levels of p53 tumor suppressor gene product, increased levels of cdc2-kinase, and increased levels of c-myc are present [48]. Although HPV-6 and -11 are "low risk" for malignant progression, the condyloma lesional milieu is conducive to proliferation, i.e., a slow-growing lesion. Elevated cdc2 kinase levels lead to elevated cdc2 protein levels, presumably allowing for higher kinase activity. Elevated cdc2 protein most probably leads to hyperphosphorylation and inactivation of pRB. The underphosphorylated (i.e., active) pRB has strong transcriptional regulatory functions and can upregulate TGF-β1, as well as other growth control factors [60]. Underphosphorylated pRB also binds and inhibits the transcription factors for the enzymes of DNA replication. With reduced active pRB, the TGF-β1 levels would drop and the transcription factors necessary to produce the enzymes of DNA replication would be upregulated, leading to unregulated hyperproliferation of cells and subsequent growth of lesions.

The above data demonstrate an increase of proliferation, but a decrease in differentiation and growth suppressive signals by the presence of "low-risk" types of HPVs. Since experimental data suggest a negative effect of HPVs on cytokine/lymphokine secretion in vitro, these changes may be due to the expression of viral genes [61]. The downregulation of TGF-β, TNF-α, and IFN-β is particularly interesting as these cytokines and others (such as GMC-SF, IL-1s, etc.) have the capacity to influence MHC class I and class II expression, and, potentially, antigen presentation [62,63].

Condylomas have very low levels of MHC class I and II mRNAs compared with uninfected skin [64]. A study by Tao et al. found that condyloma acuminatum tissue had decreased expression of MHC class I and transporter associated with antigen processing-1 (TAP-1) compared to normal tissues. TAP-1 mRNA was also decreased in condylomas. They suggested that the downregulation of MHC-I is mediated by decreased TAP-1, and that this may lead to ineffective CD8+ T-cell clearance of HPV-infected cells [65]. A significant decrease of MHC mRNA, a marker for LCs, suggests a decline of LCs [66]. Quantitative and morphologic changes of cutaneous LCs have been observed in condylomas. Since HLA-DR is expressed chiefly by LCs and keratinocytes of condylomas, the reduced numbers of LCs must cause the net reduction. Diminished levels of IL-1α and IL-1β further affect the ability of epidermal cells to present antigens, thus influencing dendritic LCs. This lack of LCs probably hampers keratinocyte presentation of antigen and leads to a decrease in the immunological surveillance.

The very low levels of IL-2 mRNAs in condylomas further suggest a significant decrease in numbers of lymphocytes [67]. Indeed, CD4 and CD8 mRNA levels are significantly lower in infected skin than in uninfected skin. Tay et al. detected a lower helper/suppressor T-cell ratio in condyloma acuminatum compared to normal tissue [68]. Other data indicate that CD8 mRNA exceeds CD4 mRNA levels in infected skin and are in agreement

with these findings [48]. This depletion of intraepithelial lymphocytes, together with the depletion of LCs, the selective depletion of CD4+ cells, and the change in the ratio of CD4+ and CD8+ subsets, support the suggestion that there is a local intraepithelial immune deficiency associated with HPV infection. This might facilitate a prolonged HPV infection and expression of other long-term effects, such as malignancy.

The presence of HPV directly or indirectly can influence MHC gene expression. A direct influence might be elicited through the expression of E7 or E5 early genes, which seem to interact with the antigen processing system in in vivo studies [69]. Ashrafi et al. showed that E5 of HPV-16 causes retention of MHC class I complexes in the Golgi apparatus, thereby preventing their transport to the cell surface. Further, they found that E5 of HPV-16 selectively downregulates surface expression of HLA-A and HLA-B, preventing their ability to present viral antigens to CD8+ T cells [70]. In a later study, they proposed that downregulation of MHC class I is a common feature of all E5 papillomavirus proteins based on the discoveries that the E5 proteins of BPV-1, BPV-4, HPV-2, HPV-6, HPV-16, and HPV-83 all serve to downregulate MHC class I [71]. Indirect effects of E7 or other HPV early gene products may be exerted through different cytokines (TGF-β, TNF-α, IL-1, etc.) or oncogenes (c-myc), which can then influence MHC class I or II synthesis [72]. Another direct effect of HPV gene products on MHC levels and antigen presentation might relate to a high abundance of early viral genes (especially E7). This differential expression can have multiple effects, leading to immunological hyporesponsiveness. First, in vitro experiments demonstrate that the HPV E7 proteins are masked in the infected cell nuclei, likely due to complex formation with cellular proteins. The consequence of this masking might be an inappropriate immune recognition, which might be the case in non-responder tumors [73]. Second, keratinocytes, which lack co-stimulatory molecules, might render E7-specific T cells anergic through peripheral tolerance [74].

Upon an active immune response, HPV-infected keratinocytes release TNF-α, which is toxic to HPV replication. Patients with more advanced cervical carcinoma in situ were found to have lower levels of TNF-α in affected areas, while in areas of normal epidermis there was constitutively expressed TNF-α from keratinocytes. Another consistent finding was the lack of expression of any adhesion or co-stimulatory molecules by epithelial LCs [75]. The lack of TNF-α, a known stimulator of LCs, may have been responsible. TNF-α also normally upregulates the expression of ICAM-1 on keratinocytes, which attracts T cells. Therefore, ICAM-1 levels may also be inappropriately low. This, in combination with the decreased numbers of LCs in the epidermis, may be contributory. Keratinocytes were, however, found to have increased expression of HLA-DR, CD 54, and CD 58, thus increasing their antigen-presenting capacity (Table 2.2) [75]. This increased expression may be futile in light of the depressed activity of LCs secondary to decreased TNF-α and other LC-activating cytokines.

The deficiency in TNF-α, with subsequent downregulation of ICAM-1 and T-cells, results in decreased IFN-γ. Hence, MHC class II is not upregulated and antigen presentation is not facilitated (Fig. 2.2). Elevations of TGF-β1, IFN-β, underphosphorylated (active) pRB, and reduced levels of cdc2 kinase and c-myc follow intralesional IFN treatment of HPV-infected sites [76], indicating a more complicated role of IFN action. The IFN causes an initial immune modulatory effect, and stimulates the immune system to overtake the infection. Thus, the cytokine and antioncogene response reflects a normal status.

Table 2.2 Alterations in Keratinocytes Following Viral Interaction

Virally-induced changes in keratinocyte structure and function
Increased expression of HLA-DR in HPV
Increased CD 54 and CD 58
Increased antigen presentation capacity
Increased ICAM-1
Increased LFA-1
Decreased TNF-*α* in condylomas (increased TNF-*α* in EV)
Decreased TGF-*β*1 in condylomas (increased TGF-*β*1 in EV)
Decreased IFN-*β* and γ
Increased cdc2-kinase
Increased c-myc
Decreased MHC-I expression
Decreased TAP-1
Increased inactive retinoblastoma tumor suppressor gene product
Decreased p53 tumor suppressor gene product

Despite the wealth of knowledge regarding the virulence and oncogenic factors of HPV, some individuals continue to be more susceptible than others to these factors. The successful clearance of HPV from the epidermis is dependent upon an intact immune system. Among immunosuppressed transplant patients, 77% eventually develop viral warts [45]. Some recent investigations found a correlation between HLA type and HPV susceptibility and immunity [77–82]. Although a critical role for HLA antigens is likely, the results vary among investigators, and the data at this point are preliminary. One study has shown strong CTL responses following T-cell exposure to dendritic cells pulsed with recombinant E7 protein [83]. This may indicate that effective antigen presentation may be the area of deficiency in those with chronic HPV infection.

T Cells

T cells are an important regulatory and effector component in SALT. Herpes simplex virus (HSV) exemplifies the role of T cells in SALT, as T cells are necessary to prevent reactivation of HSV infection. Skin-homing lymphocytes express a sialyl Lewis a- and x- closely related antigen called cutaneous lymphocyte-associated antigen (CLA) [84]. CLA is a selectin ligand that is expressed on transition of T cells from the naive to the memory status in the presence of IL-12 and TGF-β and bind E-selectin of the endothelium [85]. Therefore, for memory T cells to traffic to skin, they normally express CLA.

HSV-1 and -2 are DNA viruses with extensive cutaneous manifestations, including interactions with SALT. This leads to modulation of immunocyte subsets and the epidermal upregulation of IL-1β, TNF-α, and IL-6 [86]. HSV types 1 and 2 cause a primary infection, and then retire to their respective neuronal ganglia to asymptomatically shed viral particles. In immunocompetent individuals, HSV remains in a latent phase, with only mild or subclinical reactivations. In immunocompromised patients, reactivation is more common and severe. Orr et al. determined that inhibition of MHC-I antigen presentation to CD8+ T cells by HSV is a major factor in the ability of HSV to reactivate [87]. One important question is the role of SALT in preventing HSV reactivation.

Overcoming a cutaneous HSV infection requires intact antigen presentation and both CD4+ and CD8+ T cells [88–90]. In most viral infections, including HSV, CD8+ CTL are major effector cells, but in HSV, CD4+ cells are involved in both immune modulation and direct cell killing. Jennings et al. reported the requirement of CD4+ T cells in mounting a primary CTL response to HSV infection and identified a similar requirement for the presence of CD4+ cells in a secondary CTL response [91]. Williams et al. demonstrated that although the epidermis is the primary site of inoculation and subsequent HSV infection, LCs failed to invoke a HSV-specific proliferation of T cells from naive animals [92]. This suggests that epidermal LCs may not invoke the primary T-cell response against HSV. Instead, they acquire the ability only after maturation in an extra-epidermal site where there is sufficient cytokine stimulation. This lag in time might allow the HSV viral infection to enter the protective dorsal ganglia. Also, LCs actively invoke a secondary T-cell proliferative response to HSV, abrogated by anti-MHC class II antibodies and complement [92]. Therefore, both the primary and secondary (i.e., memory) CD4+ T-cell response to HSV requires HSV presentation in the context of MHC class II. Because T cells do not have the ability to prevent latent or recurrent HSV infection, it has been proposed that HSV alters T-cell function. Sloan and Jerome investigated this hypothesis and found that HSV-infected T cells stimulated through the T-cell receptor selectively secreted IL-10, thereby favoring viral replication and suppressing cellular immunity. Further, they found that activation of p38 was necessary for IL-10 secretion by infected T cells [93].

The CD4+ Th cells have been categorized into two major subsets, Th1 and Th2 [94]. Th1 cells mainly secrete IL-2, IFN-γ, and TNF-α, while Th2 cells mainly secrete IL-4, IL-5, and IL-10. Interestingly, the cytokine activity of each Th subset downregulates the activity of the other subset. Th1 cells are generally efficient in controlling viral and intracellular pathogens, while Th2 cells better control bacterial and parasitic infections by augmenting humoral immunity. The presence of Th1 and Th2 differences in skin and their involvement in cutaneous disease have been demonstrated [95]. HSV lesions demonstrate a prevailing Th1 pattern in which the induction of MHC-II on epidermal cells and the activation of CD8+ T cells through IL-12 and IFN-γ are particularly important in the control of recurrent infections (Fig. 2.7) [96]. Zhao et al. found that $CD11c^+$ dendritic cells containing viral peptides in the form of MHC-II molecules stimulated HSV-specific CD4+ cells to secrete IFN-γ. Further, they found that only $CD11b^+$ submucosal dendritic cells presented antigens to CD4+ cells and induced IFN-γ secretion. LCs and $CD8\alpha^+$ dendritic cells did not contribute to the Th1 immune response [97].

Thus, the ideal HSV vaccine would induce both neutralizing antibodies and a Th1 immune response. HSV virions consist of a core containing viral DNA, surrounding tegument proteins, and an outer envelope containing numerous glycoproteins (Fig. 2.8). Glycoproteins D (gD) and B (gB) have been used with some success in vaccine studies [96]. Administration to mice of HSV-2 plasmid vaccines encoding the gD protein, as well as the Th1 cytokines: IL-1, IL-12, IL-15, and IL-18, resulted in immunity to subsequent challenge with HSV-2. However, mice inoculated with plasmids encoding the same HSV-2 gD protein, but with Th2 cytokines, IL-4 and IL-10, had increased morbidity and mortality [37]. If the Th2 cytokines downregulate the Th1 response, decreased cellular immunity, the primary defense against cutaneous herpes infection is experienced. Stanberry et al. found that a HSV-2 gD subunit vaccine was 73–74% effective in preventing HSV-2 in women who were seronegative for both HSV-1 and -2. They proposed that the vaccine was ineffective in men because men have decreased Th1 immune responses compared to women [98]. Other vaccine-based studies have shown that vaccination with HSV-2 DNA plasmid vaccines favors a Th1 response, whereas induction of immunity with recombinant protein gD induced a Th2 response. While both were protective from lethal challenge, only the plasmid DNA vaccine induced a response that was protective against subsequent herpetic lesions and HSV-induced morbidity [99]. While CMI is most relevant to cutaneous manifestations, the humoral response offers protection from HSV encephalitis and HSV-induced mortality [36,99].

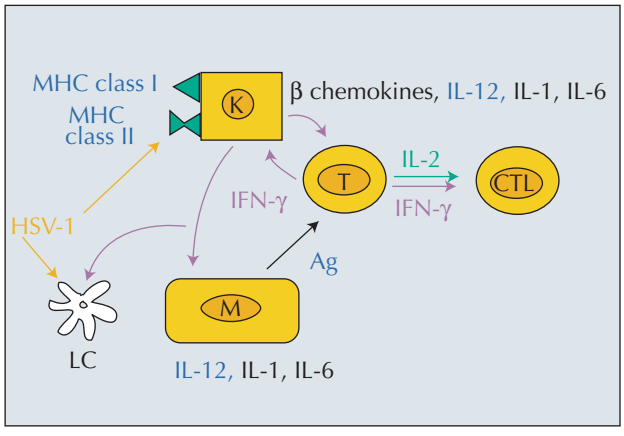

Figure 2.7 Immune processes in the recurrent HSV lesion: in vivo secretion of cytokines and chemokines in herpetic lesions. Ag, antigen; CTL, cytotoxic T lymphocytes; IFN, interferon; IL, interleukin; K, keratinocytes; LC, Langerhans cell; M, macrophage; MHC, major histocompatability complex. (Reprinted from AL Cunningham, Z Mikloska. The holy grail: immune control of human herpes simplex virus infection and disease. Herpes 8 (Suppl 1): 7A, 2001.)

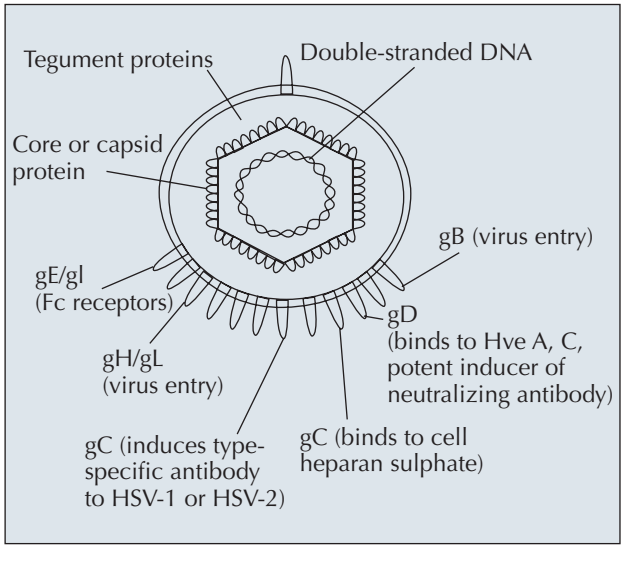

Figure 2.8 Structure of HSV virions: g, glycoprotein; HSV, herpes simplex virus; Hve, herpes virus entry mediator. (Reprinted from AL Cunningham, Z Mikloska. The holy grail: immune control of human herpes simplex virus infection and disease. Herpes 8 (Suppl 1): 8A, 2001.)

Table 2.3 Alterations in T Cells Following Viral Interaction

Virally-induced changes in T cell structure and function
Increased IL-10 in HSV
Increased activation of p38 in HSV
Increased IFN-γ following antigen presentation by dendritic cells
Increased Eta-1 in cell-mediated immune response to HSV
Decreased γδ + T cells in HIV

Eta-1 or osteopontin, a newly described cytokine, further supports the role of CMI in HSV-1 infection. Eta-1 is a necessary component of CMI [100]. Without this cytokine, IFN-γ and IL-12 do not increase appropriately and IL-10 rises inappropriately. Eta-1 –/– mice infected with HSV-1 were unable to mount a delayed-type hypersensitivity (DTH) reaction when further inoculated in the foot pad with HSV-1. Eta-1 +/+, HSV-1 infected mice displayed a strong DTH response in the foot pad when inoculated with HSV-1 (Table 2.3). Use of the antibody against Eta-1 results in similar findings. Although this study does not deny a role for Th2 and the humoral response for HSV protection, it emphasizes the overwhelming significance of CMI.

Endothelial Cells

The endothelium in many respects is the gate keeper of the skin, only allowing certain cells and components through. Adhesion molecules expressed by endothelial cells play a significant role in leukocyte entry into skin. E-selectin, P-selectin, ELAM-1, ICAM-1, ICAM-2, and VCAM-1 are the predominant adhesion molecules of endothelial cells used for interaction with leukocytes. The first part of this interaction involves the slowing of blood flow and the margination of cells towards the periphery of the vasculature near the endothelium. Selectins mediate the next step known as rolling, which consists of loose and transient associations between the white cells and the endothelium. This is followed by the firm and stable adhesions, formed by integrins such as ICAM-1, ICAM-2, and VCAM-1. The final step is diapedesis, mediated in part by ICAM-1 [101]. Through this process, endothelial cells are an important element in the eradication of pathogens. However, certain pathogens show tropism for endothelial cells and can infect them. Cytomegalovirus (CMV) is a virus well documented to have endothelial involvement (Fig. 2.9). Wang and Shenk determined that CMV tropism for endothelial cells is the result of a complex of viral glycoproteins, gH and gL, which are known to be involved in viral entry and fusion, with CMV genes pUL128, pUL130, and possibly pUL131A [102,103]. Although CMV has numerous mucocutaneous manifestations, especially in immunocompromised patients, the majority of research on endothelial involvement of CMV has been carried out on extracutaneous tissues.

CMV infection of endothelial cells directly affects transendothelial migration of white cells and further dissemination of the CMV virus. Studies with human umbilical vein endothelial cells (HUVEC) have shown that CMV infection upregulates endothelial expression of E-selectin, ELAM-1, ICAM-1, and VCAM-1 [104,105]. ELAM-1 and ICAM-1 caused increased adhesion of polymorphonuclear (PMN) cells and T-lymphocytes, while VCAM-1 resulted in increased adhesion of monocytes and T-lymphocytes to

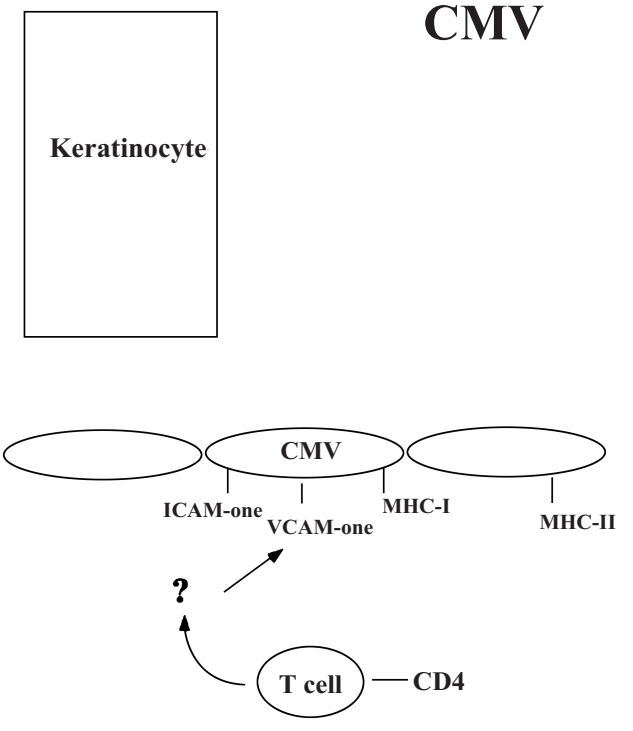

Figure 2.9 CMV is disseminated to healthy tissue through endothelial infection. (1) CMV causes endothelial upregulation of vascular adhesion molecules by a paracrine mechanism; (2) transendothelial migration by white cells results in infection from adjacent endothelial cells; (3) infected white cells disseminate CMV into healthy tissue.

endothelial cells [104]. IL-1β may mediate the increased expression of these endothelial surface antigens. CMV infection of endothelial cells results in increased secretion of IL-1β, which by a paracrine route affects adjacent non-infected endothelial cells. Significantly, studies have shown that certain white cells, such as neutrophils, can become infected during transmigration across infected endothelial cells and subsequently disperse this infection to other cells [106,107]. This can tremendously increase the dissemination of the virus and the inflammatory response throughout the body.

Endothelial cells also express MHC class I and II antigens, subsequent to IFN-γ [108]. In the past few years, a host of other escape mechanisms for CMV have been proposed and many of these involve CMV's influence on MHC class I and II expression by the endothelium. In one study, it was shown that CMV disrupts the signal transduction pathway that normally results in expression of IFN-α [109]. Specifically, CMV decreases the expression of Janus Kinus 1 and p48, important signal transducers involved in the expression of IFN-α. Decreased IFN-α, a key antiviral cytokine, secondarily results in MHC-I, IFN regulatory factor-1, MxA, and 2',5-oligoadenylate synthetase gene expression in fibroblasts and endothelial cells infected with CMV. Similarly, another group showed that CMV-infected arterial and venous endothelial cells are refractory to upregulation of MHC-II by IFN-γ [110]. CMV has the ability to interrupt signal transduction of the JAK/STAT pathway, which is induced by IFN-γ and normally upregulates MHC-II [111]. The combination of these two findings results in decreased expression of both class I and II antigens by the endothelium. The result is an impaired ability to induce an immune response and clear CMV infection. Only with

a competent immune system can important molecules be sufficiently upregulated allowing for effective antigen presentation and immunoresponsiveness.

In a later study, Kas-Deelen et al. determined that CMV leads to enhanced expression and activity of the ecto-ATPase and ecto-5'-nucleotidase enzymes on endothelial cells, which in turn leads to increased adenosine production. They hypothesized that these enzymes are upregulated to offset the procoagulatory effects of CMV. They also found that production of oxygen radicals by PMN cells was decreased in the presence of CMV-infected endothelial cells, which they suggest is a result of increased adenosine production [112]. A related CMV escape mechanism was elucidated by Zandberg et al. They found that the expression of P2 purinergic receptors on endothelial cells was upregulated in CMV-infected cells, but not in uninfected cells or HSV-infected cells. They proposed that these receptors may facilitate rapid hydrolysis of adenosine triphosphate and adenosine diphosphate leading to anti-inflammatory and anti-aggregatory conditions, which may allow CMV to enter endothelial cells [113].

HIV infection also affects the endothelial component of SALT. HIV-infected cells release many different cytokines with TNF-α being the most common [114]. TNF-α contributes to endothelial leakiness by: (1) induction of cytokine release; (2) expression of adhesion molecules; and (3) direct enhancement of endothelial permeability. Furthermore, the HIV-derived transactivator (tat) protein, which is secreted into extravascular tissue, directly stimulates endothelial cells to express E-selectin, ICAM-1, VCAM-1, and ELAM-1 [115–117]. The molecules are necessary for endothelial trapping of leukocytes in the vasculature. IL-6 synthesis, enhanced by tat, increases endothelial permeability, which facilitates leukocyte passage out of the vasculature [118]. This aids in the dissemination of infected cells into virus-free tissue. This may also be deleterious to epithelial homing of leukocytes and contribute to epidermal depletion of LCs.

Interactions between the HIV tat protein and the endothelium may be responsible for the highly aggressive behavior of AIDS malignancies. As mentioned earlier, increased expression of VCAM-1, ICAM-1, ELAM-1, and $\alpha V\beta 3$ integrin, as well as other vascular adhesion molecules is thought to be a direct consequence of the tat protein. These proteins increase cellular motility and transendothelial migration. It has been shown that the tat protein increases the motility of cells from the AIDS-related Burkitt's lymphoma cell lines and AIDS primary effusion lymphoma (PEL) cell lines. Tat not only enhances the migration of lymphoma cells, but increases their adhesion to endothelial cells. This study gives one explanation for the malignant behavior of non-Hodgkin's lymphoma (NHL) in patients with AIDS. Interestingly, antibodies against VCAM-1 inhibited this increased motility. Other actions of tat have also been studied and described.

Human herpes virus-8 (HHV-8), discovered by Chang in 1994, is the causative agent of Kaposi's sarcoma (KS). However, the increased incidence of KS in AIDS patients may be partially related to the HIV tat protein. One mechanism by which the tat protein has been shown to act is mobilization of b-fibroblast growth factor (b-FGF) [116]. Heparin sulfate proteoglycans normally provide binding sites for b-FGF. Tat competes for these sites and increases the concentration of free b-FGF. b-FGF, a well-known angiogenic factor, may act synergistically with the tat protein and HHV-8 in the development of KS [118]. Another study found that tat protected three KS cell lines and HUVECs from apoptosis induced by the chemotherapeutic agent vincristine and serum starvation, respectively. Tat upregulated Bcl-X_L expression, leading to a decrease in caspase-3-mediated apoptotic activity in the vincristine-treated KS cells [119]. Sivakumar et al. discovered that HHV-8 induced vascular endothelial growth factors (VEGF) A and C within 30 minutes of infection of human microvascular dermal endothelial cells (HMVEC-d). They suggest that VEGF A and C may subsequently cause angiogenesis and lymphangiogenesis, respectively, thereby facilitating growth of KS lesions [120]. In a later study, they found that HHV-8 causes the formation of a complex of integrins, including $\alpha V\beta 5$, $\alpha V\beta 3$, and $\alpha 3\beta 1$, with amino acid transporter protein xCT and glycoprotein CD98, which may facilitate viral entry into HMVEC-d (Table 2.4). Further, they determined that the CD98-xCT complex may mediate viral gene expression in the post-entry stage of infection [121].

Table 2.4 Alterations in Endothelial Cells Following Viral Interaction

Virally-induced changes in endothelial cell structure and function
Increased E-selectin in HIV and CMV
Increased ELAM-1 in HIV and CMV
Increased ICAM-1 in HIV and CMV
Increased VCAM-1 in HIV and CMV
Increased cellular motility and transendothelial migration
Increased IL-1β in CMV
Decreased IFN-α in CMV
Decreased MHC-I and -II in CMV
Increased adenosine production in CMV
Increased expression of P2 purinergic receptors in CMV
Increased IL-6 in HIV
Increased $\alpha V\beta 3$, $\alpha V\beta 5$, and $\alpha 3\beta 1$ integrins
Increased b-FGF in HIV/HHV-8 co-infection
Increased VEGF A and C in HHV-8

Conclusion

SALT is comprised of keratinocytes, LCs, skin tropic T cells, and lymphatic endothelial cells of the skin. The epidermis, which is involved in many viral infections, contains all of the components needed for an effective immune response: antigen-presenting LCs, T cells, and cytokines from leukocytes and keratinocytes. There have been some recent advances in the study of the cutaneous immunology involved in infections with HIV, HPV, HSV (Fig. 2.10), and CMV. In general, viral diseases with cutaneous manifestations lead to a decline in epidermal LC numbers, which is probably a reflection of the LCs emigration out of the epidermis and entry into regional lymph nodes. These events lead to LCs activation and antigen presentation to T cells. In HSV, there is subsequent T-cell infiltration of the epidermis, comprised of CD4+ cells that have both immune modulatory action and direct cytotoxic action. In HIV, where there is a systemic depletion of CD4+ cells, the epidermis is left with reduced numbers of T cells. Intradermal injection of IL-2, however, leads to an epidermal cellular infiltration in HIV-positive individuals. In HPV-induced condyloma, intralesional IFN increases LCs, CD4+, CD8+ cells in the skin, as well as TGF-β1, TNF-α, pRB, and p53. Therefore, viral infections

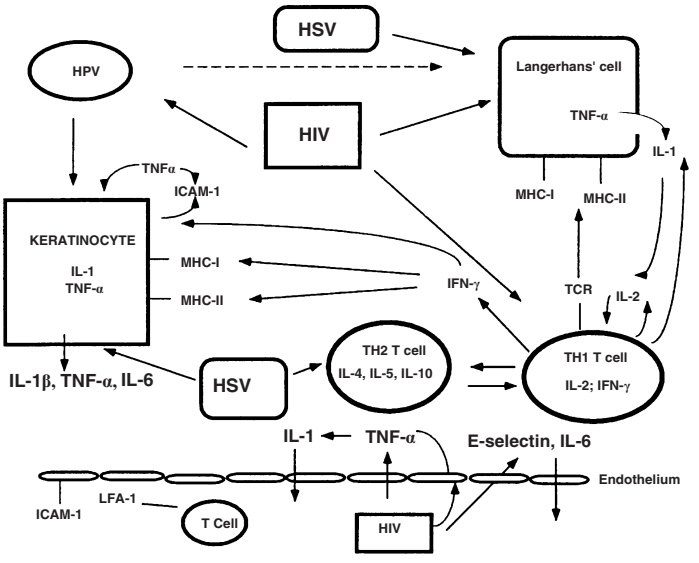

Figure 2.10 The presumed role of SALT in cutaneous viral infections.

involving the epidermal immune system have certain similar characteristics, while other parameters are unique to the infecting virus. The immune system resident in the epidermis is significantly affected by infections with CMV, HIV, HPV, and HSV. SALT is able to eradicate a cutaneous viral infection and retain memory of the infection to ward off future infections. In certain instances, SALT does the opposite and enhances the pathology of a cutaneous viral infection. One common feature in HIV, HPV, and HSV infections is reduction in epidermal LC counts, but this does not indicate a common mechanism. For example, HIV is known to infect LCs and have cytopathic effects, while this has not been shown for HPV or HSV. Possibly, viral infections of the epidermis require the presence of LCs only in extra-epidermal sites (e.g., lymph nodes), where antigen processing takes place. The above-mentioned viruses affect epidermal T-cell counts and subset proportions differently and may indicate the differential cytokine levels or patterns of expression that might exist in different viral infections. Although much has been learned about cutaneous viral immunology during the past few years, further studies are needed to enhance our understanding of SALT in viral infections.

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3 Poxviruses

Jessica Clark and Dayna Diven

INTRODUCTION TO POXVIRIDAE

The poxvirus family affects both humans and animals. The poxviruses are the largest of all animal viruses and are easily visualized on light microscopy. When seen under the electron microscope, the poxviruses are brick-shaped or oval 200–400 nm structures. The nucleosome contains double-stranded DNA surrounded by a membrane. The outer surface of the lipoprotein bi-layer has randomly arranged surface tubules, which give the virion its characteristic textured appearance. The lipid composition of the membrane is different from that of the host cell membrane [1]. The nucleoprotein core, lateral bodies, and membrane constitute an infectious collective unit. The virus may also acquire an envelope (Fig. 3.1).

Replication occurs autonomously in the cytoplasm of cells. After uncoating, the virion produces early enzymes and early virion proteins and late enzymes and late virion proteins [1]. These replication "factories" are independent of the host nucleus and are discernable on light microscopy as basophilic staining B-type inclusion bodies. The genome undergoes spontaneous recombination.

Numerous strategies are used by the poxviruses to evade the host immune system. These include production of homologues of mammalian tumor necrosis factor receptor, interleukin-1 beta-receptor, interleukin-18 binding protein, interferon-alpha/beta receptor, and interferon-gamma receptor, as well as a complement-binding protein and a caspase inhibitor. These proteins are thought to inhibit the function of cytokines and complement proteins, neutralizing the host's antiviral response [2].

The toxic effect of poxviruses causes cell rounding and clumping, degeneration of cell architecture, and the production of cytoplasmic vacuoles. Depending on the poxvirus, clinical presentations can include a localized, self-limited infection by inoculation to the skin (e.g., ORF) or a fulminant systemic disease

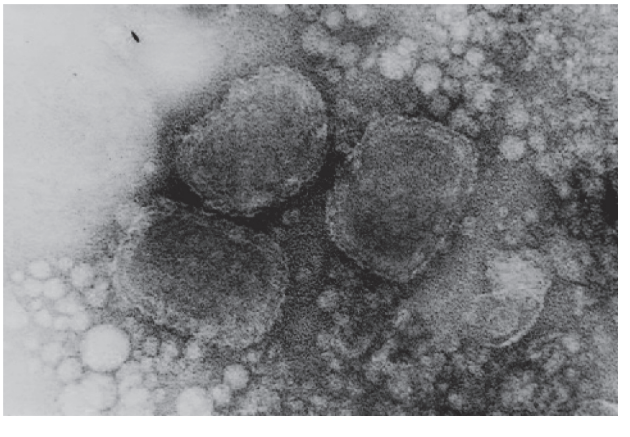

Figure 3.1 Poxvirus (vaccinia) brick-shaped to oval virus particles with electron-dense DNA core and visible outer membrane are seen in the vacuolated keratinocyte cytoplasm (× 20, 160). (Photograph courtesy of Harvey Blank, M.D., Department of Dermatology, University of Miami School of Medicine, Miami, FL.)

Table 3.1 Hosts and Portal of Entry for Selected Poxvirus Species

Genus	Species	Hosts	Portal of entry (for Humans)
Orthopoxvirus	Vaccinia virus	Humans	Skin
	Cowpox virus	Humans, cats, cattle	Skin
	Variola virus	Rodents, humans	Respiratory tract
	Monkeypox virus	Humans, monkeys, rodents	Skin
Parapoxvirus	Orf virus	Goats, sheep, camels, humans	Skin
	Bovine papular stomatitis virus	Cattle, humans	Skin
	Pseudocowpox virus	Cattle, humans	Skin
	Red deer poxvirus	Red deer	
Avipoxvirus	FowlPox virus	Birds	
Capripoxvirus	Sheep-Pox virus	Sheep, goats, cattle	
Leporipoxvirus	Myxoma virus	Squirrels, rodents, rabbits	
Suipoxvirus	Swinepox virus	Swine	
Molluscipoxvirus	Molluscum contagiosum virus	Humans (chimpanzees)	Skin
Yatapoxvirus	Tanapox virus	Humans, monkeys	Mosquitoes suspected
	Yabapox	Humans, monkeys	Skin

*Adapted from Buller and Palumbo [1], 1991, Tables 1 and 3, p. 82 and 98.

TAXONOMY OF VIRUSES

Poxviridae family
Chordopoxvirinae subfamily
Orthopoxvirus genus (vaccinia, cowpox, variola, monkeypox)
Parapoxvirus genus (orf, bovine papular stomatitis, pseudocowpox)
Yatapoxvirus genus (tanapox)
Molluscipoxvirus genus (molluscum contagiosum)

WORLDWIDE GEOGRAPHICAL INCIDENCE

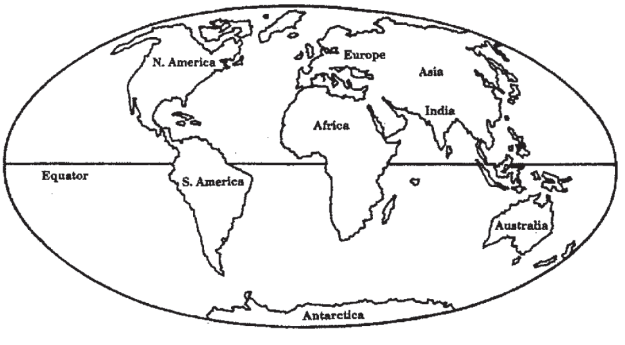

Figure 3.2 Taxonomy and incidence of poxviruses.

(e.g., variola). Different species can demonstrate a wide range of signs and symptoms from the same virus. Other poxviruses, like molluscum contagiosum, cause localized cell proliferation. Variola virus, the causative agent of smallpox, and molluscum contagiosum virus (MCV) are the only two known poxviruses to cause disease exclusively in humans [2] (Table 3.1).

Poxviruses that are not known to infect humans, including camelpox and sheep and goat lumpy skin disease complex, may result in great economic hardship in dependent farming communities. Our review includes only those poxviruses that infect humans (Fig. 3.2).

ORTHOPOXVIRUS INFECTIONS

Smallpox

Definition

An infection caused by the variola virus that affects only humans.

History

The history of the rise and fall of the smallpox virus is both fascinating and unique. The saga includes centuries of death and disfigurement followed by scientific triumph, although the fate of the virus remains undecided. For generations, the interaction of smallpox and humans has been characterized by unparalleled persistence and diffusion [5]. Smallpox is thought to have originated in Africa with subsequent spread to India and China thousands of years before Christ. The first recorded smallpox epidemic was in 1350 BC during the Egyptian-Hittie war [5]. Spread to Europe was evident between the 5th and 7th centuries. Smallpox was documented in the West Indies in 1507 and followed the Spanish conquest into the New World [6]. The immunity of the Spanish troops and the susceptibility of the peoples of Mexico and Peru to smallpox may have been a factor in the outcome of that conquest. During the 17th and 18th centuries, epidemics occurred in the North American colonies [6]. At one time, smallpox was endemic throughout the world, except in Australia and other small islands [6]. Large-scale epidemics caused millions of deaths in Europe and Mexico [5].

In the 1700s, the observation that smallpox survivors were immune to future outbreaks led to the practice of variolation in China, India, and Turkey. Variolation is the deliberate inoculation of an uninfected person with the smallpox virus by contact with the pustular lesion as a prophylaxis against a more severe form of smallpox. Lady Mary Wortley Montague, herself a smallpox survivor, is credited with advancing the smallpox variolation in England [7,8]. In the late 18th century, Edward Jenner, acting on reports of smallpox immunity by milkmaids who had developed cowpox, developed the first smallpox vaccine. The Council of the Royal Society rejected his idea. Jenner ended up self-financing the publication, and the vaccine has been used for over 200 years [5].

The decline of smallpox during the 20th century is correlated with a rise in smallpox vaccination. In the latter half of the 20th century, other countries including Africa and Asia continued to suffer major disease outbreaks, whereas most of North America, Western Europe, Australia, and New Zealand were free of the disease. In 1967, the World Health Organization (WHO) set forth a worldwide campaign to eradicate smallpox. By 1976, only Ethiopia and surrounding areas were still affected by the disease. On May 8, 1980, the World Health Assembly declared the world free of smallpox [8]. The spread of smallpox evolved over thousands of years, the global spread occurred for hundreds of years, and its eradication was sealed 13 years after the WHO program was initiated.

Incidence

In the USA, the last outbreak of smallpox occurred in Texas in 1949 (eight cases, one death) [9]. The last endemic case of smallpox occurred in Somalia in October 1977 [9]. A laboratory-associated outbreak was reported at a university in England in 1978 [9,10]. The infected person worked on a floor above the laboratory and died 1 month after infection [10]. By 1984, all countries had discontinued vaccination of the general population and they did not require travelers to certify vaccination [11] (Fig. 3.3).

Routine vaccination in the USA continued until 1971. The vaccine was given sporadically after this until 1983, when vaccine producers were urged to reserve the vaccine for military personnel only [12]. In 1986, it was recommended that military personnel no longer be vaccinated [13]. Smallpox vaccination was officially discontinued in US military recruits, except for special units, in 1990. In 2000, however, fears of biological

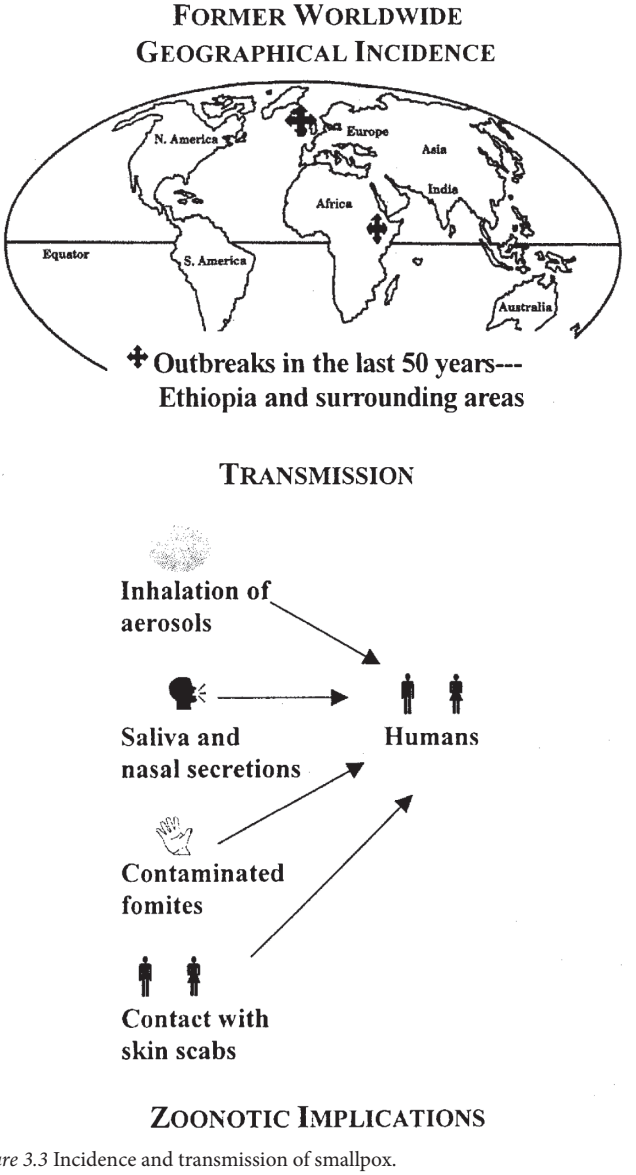

Figure 3.3 Incidence and transmission of smallpox.

warfare led to renewal of vaccine production for use by the military. In January 2003, the Department of Defense initiated vaccination of selected military forces and essential deployed civilians and contractors [14]. A 2007 Department of Defense release reports that over 1,200,000 operational forces and healthcare workers have been vaccinated against smallpox with adverse event rates below historically reported rates [14].

The WHO has investigated rumors of smallpox, all of which turned out to be misdiagnosed varicella or other skin disease. The CDC and the Russian State Center for Research on Virology and Biotechnology in Koltsovo are the only laboratories known to house the smallpox virus [15]. In this post-eradication era, it still remains a possibility that unsanctioned laboratories are storing variola viruses. Due to increased threats of bioterrorism, physicians should familiarize themselves with the signs and symptoms of this infection.

Pathogenesis/Epidemiology

The spread of smallpox usually occurs through intimate contact via the respiratory route [1]. Infection can develop from inhalation of aerosolized virus, contact with saliva or nasal secretions, contact with skin crusts, or through contaminated fomites, such as bedding [17].

On the third to fourth day after infection, an asymptomatic viremia develops with continued viral production in the spleen, bone marrow, and lymph nodes. Fever and toxemia follow on day 8. The virus goes on to infect adjacent cells of the dermis and oral mucosa. Death most likely results from the toxemia produced by circulating immune complexes and soluble variola antigens [18].

Infected people are contagious from the onset of illness until the last crusts of the lesions are gone, although infectivity is greatest in the earlier stages of disease. The rate of infectivity for susceptible contacts is 30% [19]. Population density and immunity affect the extent of spread. Eradication was obtainable because there are no known animal reservoirs for smallpox. Also, no significant subclinical carrier state exists [6]. Generally, environmental conditions do not affect the virility of the virus.

Infection is observed only in humans. However, current research has elicited infection in macaques. This development is exciting as the macaques may be a source to learn more about the virus [20].

Clinical Manifestations

Currently, the clinical diagnosis of smallpox is based on several criteria. The major criteria are (1) a febrile prodrome 1–4 days before rash onset; (2) the classic smallpox lesions (i.e., deep-seated, firm, round, well-circumscribed vesicles); and (3) lesions that are at the same stage of development (Figs. 3.4 and 3.5). The minor criteria include (1) a centrifugal distribution of lesions, with the first lesions on the oral mucosa or palate, face, or forearms; (2) a toxic or moribund appearance; (3) the slow evolution of lesions of 1–2 days per stage; and (4) lesions that appear on the palms and soles [21] (Table 3.2).

Variola major, the severe form of smallpox infection, can cause pulmonary edema from heart failure, leading to death. The case fatality rate for variola major ranges from 30–40% [22]. Variola minor (alastrim) is a milder form of the illness and results in few fatalities. Although the pathogenicity varies tremendously, the two different strains of variola only differ by 2% of their genome [22].

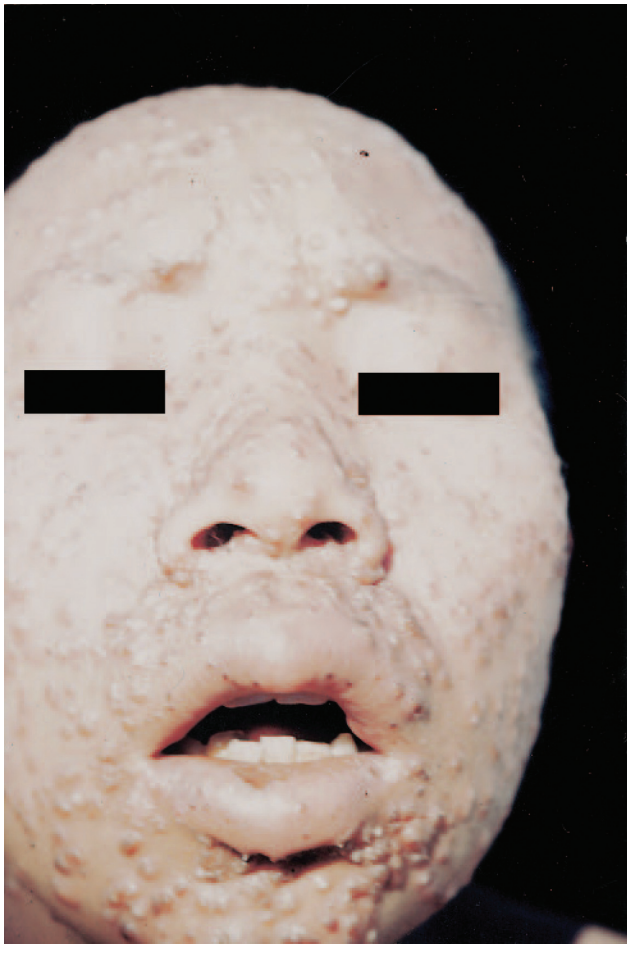

Figure 3.4 Smallpox in a Chinese soldier. (Photograph courtesy of Harvey Blank, M.D., Department of Dermatology, University of Miami School of Medicine, Miami, FL.)

The four types of variola major presentations include (1) ordinary, (2) modified (by previous vaccination), (3) flat (also known as malignant), and (4) hemorrhagic, with the latter two having the highest fatality rates. The majority of cases in unvaccinated individuals were of the ordinary or classic type. Case reports of modified smallpox in previously vaccinated individuals were mild and did not result in mortality. The malignant and hemorrhagic forms of smallpox were attributed to host factors, such as a deficient cellular immune response to the virus. The malignant or flat form of variola was more common in children, progressed more slowly than the classic presentation, and did not produce pustules. The mortality rate for this form approached 100%. The hemorrhagic variety of variola is characterized by mucosal and skin hemorrhage. It was reported more commonly in pregnant women and carries a high mortality rate of 90–100% regardless of vaccination status. Of note, pregnant women who contracted smallpox had a 3–4 times higher case fatality with any form of smallpox compared to men and non-pregnant women of the same age, possibly linked to a T-helper type 2 immune response associated with pregnancy [22].

The incubation period for the variola virus is 12–13 days. Those who survive often have significant scarring from pustules and granulation tissue formation. Corneal infection frequently results in blindness (Table 3.3).

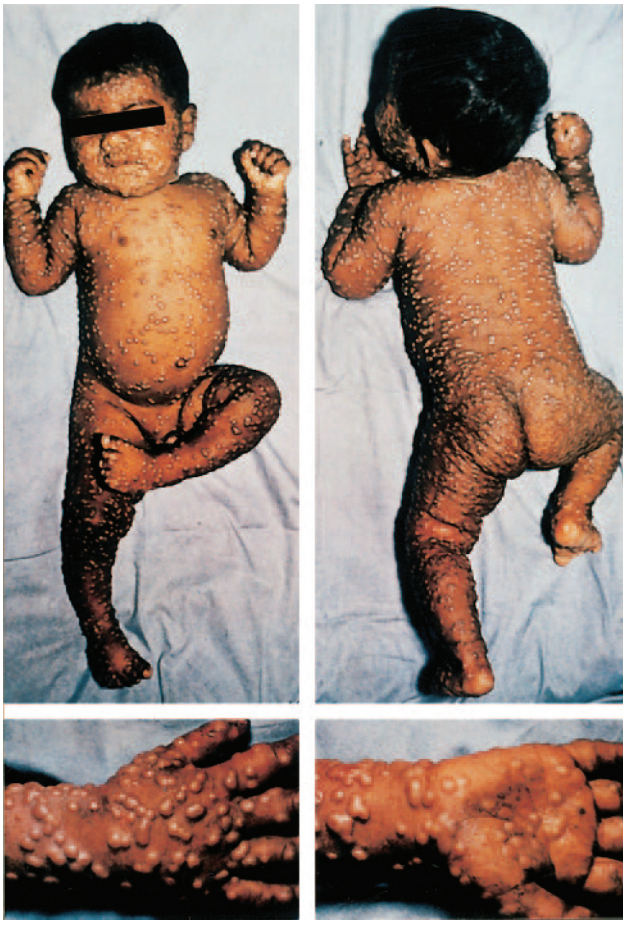

Figure 3.5 Smallpox in an Indian baby. (Photograph courtesy of the World Health Organization, Geneva, Switzerland.)

Dermatopathology

Ballooning degeneration and cytoplasmic inclusion bodies (Guarnieri bodies) within keratinocytes are observed. Reticular degeneration and dermal hemorrhage ensue, with massive polymorphonuclear cell infiltrates. This is followed by crusting and new epithelial formation [23].

Laboratory Findings

Laboratory confirmation may be obtained from silver impregnation or fluorescent antibody staining of smears taken from skin lesions [6]. However, a negative smear does not exclude the disease. A laboratory designed to handle the virus uses chick embryo or tissue culture for identification. A fourfold or greater rise in antibody titer is diagnostic. Electron microscopy can also be used to identify the virus.

Treatment/Prophylaxis

If the diagnosis of smallpox is considered, immediate isolation of the patient is in order, after which the Centers for Disease Control (CDC) should be contacted. All patient contacts should be identified. Supportive care and treatment of bacterial infections are the mainstays of treatment (Table 3.4). The current population of the USA is considered immuno-naive to the variola virus with over half of the population never vaccinated and the remainder with unknown titers of waning immunity [20].

Future Considerations

The smallpox virus is a potentially dangerous agent of biological terrorism. Infection may be caused by only a few virions [18]. The fear is significant because a large portion of the world's population is not immune to smallpox, no effective treatment exists, and the secondary attack rate is 25–40% with a case fatality rate of 30% [16].

There is ongoing debate regarding whether all stocks of the variola virus should be destroyed. Opponents of destruction argue that more scientific inquiry requiring the whole virus could be done to identify the virulence segment of the genome [24–27]. Opponents also believe that variola's unique host specificity makes it valuable for future research and that completely destroying the virus would set a bad precedent. Additionally, they argue that specimens collected during epidemics could still be in

Table 3.2 Clinical Manifestations of Smallpox Infection

Time after exposure	Clinical manifestations	Laboratory analyses	Other notes
12–13 days	Prodrome of fever, malaise and backache—lasts 3–4 days ↓ Exanthem appears and quickly evolves:	Silver impregnation or fluorescent antibody stain of skin lesion smears;* electron microscopy; chick embryo or tissue culture (reserved for specialized laboratories); fourfold increase in antibody titer	Portal of entry—respiratory tract Infectivity is maximal during the first week of rash
14–16 days	↓ macules ↓ papules ↓ Vesicles		Initial eruption on palms of hands and soles of feet; distribution is centrifugal and on extensor surfaces All lesions are in a similar stage at any one time
19–20 days	↓ Pustules		
25–27 days	↓ Crusts (see Fig. 3.5), new epithelial formation		Overall mortality rate is 30% Scarring may occur; corneal infections may result in blindness Scarring may be severe

*A negative smear does not exclude disease.

Table 3.3 Differential Diagnoses of Smallpox

Varicella (chickenpox): Small delicate vesicles, concentrated on trunk, face, and flexor extremities, with rare deep scarring. Lesions are generally in various states of development
Syphilis: Resembles early smallpox but does not progress as smallpox does
Monkeypox: Has a greater tendency to produce both lymphadenopathy and skin lesions in "crops"

existence and that similar viruses, such as monkeypox, could mutate [24–27].

Those who want the virus destroyed argue that the genomes of reference strains have been cloned and sequenced. Also, monkeypox virus DNA is easier to study than variola virus in that it is similar to variola, has an animal host, and requires less stringent laboratory precautions [16]. In mid-1999, the World Health Assembly recommended a delay in the destruction of known smallpox reserves. The WHO was directed to appoint a new group of experts to establish what research, if any, had to be carried out to reach a global consensus on the timing for the destruction of existing variola virus stocks. This action permits further research into antiviral agents, improved vaccines, genetic structure analysis, and pathogenesis of smallpox. The World Health Assembly authorized continued postponement of destruction of the virus in 2002 [28]. By mid-2002, the USA ordered 209 million doses of smallpox vaccine [29]. In 2005, the World Health Assembly continued to work on increasing global smallpox vaccine reserves [28].

Conclusion

For centuries, smallpox terrorized the civilized world and affected millions of people [5]. Worldwide eradication of this virus is a unique event in history. Controversy still exists regarding the fate of the remaining stores of virus [26,27,30].

Vaccinia

Definition

An orthopox virus that affects a wide range of vertebrate hosts. Vaccinia virus is the constituent of the smallpox vaccine.

History

Vaccinia virus is the best studied poxvirus. Edward Jenner first used cowpox in 1796 [4]. It has been used for over 200 years as a vaccine for smallpox. The virus was originally thought to be isolated from infected cows and, later, horses [31–33]. However, it is now considered to be a laboratory virus with no natural reservoir [4]. In 1972, routine vaccination in childhood was discontinued in the USA. In early 2003, smallpox vaccination of selected US military forces and emergency healthcare civilian personnel was initiated [14]. The vaccine is currently given to select military recruits and civilian first responders [34].

Table 3.4 Symptomatic Treatment of Smallpox

Secondary symptoms	Treatment
Fever	Antipyretics
Secondary bacterial infection of open lesions	Systemic antibiotics
Pulmonary edema	Morphine, oxygen, intravenous loop diuretics, afterload reduction, inotropic support, aminophylline

Incidence

Infection with vaccinia virus occurs only in laboratory workers and after smallpox vaccinations in patients with atopic dermatitis (eczema herpeticum) who are either vaccinated themselves or exposed to family members getting smallpox vaccinations. The vaccine strain is considered to be a relatively safe virus because it does not cause serious disease in immunocompetent humans or animals [35] (Fig. 3.6).

Pathogenesis

The vaccinia virus is introduced into the outer layers of skin. Fifteen punctures are performed with a bifurcated needle in the deltoid region [36]. A localized infection occurs owing to the host immune response. Although immunity is induced through antibody and cell-mediated responses, the T-cell response appears to be necessary for full protection. This has been noted with observation of total immunity in children with agammaglobulinemia. New evidence suggests that relevant immunity may last for up to

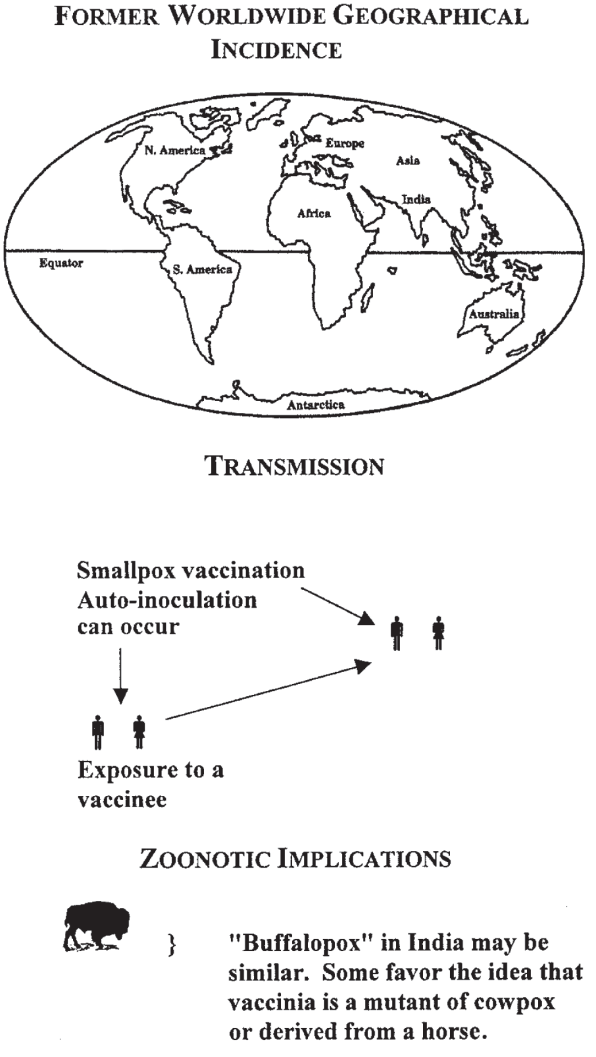

Figure 3.6 Incidence and transmission of vaccinia.

75 years after vaccinia vaccination, instead of only 3–5 years as previously thought [37].

The vaccinia vaccine induces neutralizing antibodies that are also protective for other orthopox viruses (monkeypox, cowpox, and variola). Symptoms and severity of smallpox disease may be decreased with administration of vaccinia vaccine within the first days after initial exposure [36]. Laboratory employees do not require routine vaccination if working with highly attenuated strains of vaccinia [36].

Clinical Manifestations

The vaccine causes a local reaction by multiplying in the basilar epithelium. A papule occurs 2–3 days after vaccination with a bifurcated needle multiple puncture technique. A successful primary vaccination produces a major reaction characterized by the appearance of a Jennerian pustule on day 7 [18] (Figs. 3.7 and 3.8). In the past, vaccination scars were used with considerable accuracy to assess the vaccination status of the individual or of populations [38] (Table 3.5).

Swelling and tenderness of regional lymph nodes can occur 3–10 days after vaccination and can persist up to 4 weeks after healing of the vaccination site. Other common reactions include local satellite lesions, local edema, and intense inflammation similar in appearance to bacterial cellulitis (Figs. 3.9–3.15). Systemic symptoms can also occur in healthy individuals. Thirty-six percent of adults reported feeling malaise enough to miss work, school, or recreational activities, or to have sleeping difficulties [39] (Table 3.6).

Complications of Vaccination

Rarely, healthy patients can experience a generalized vaccinia, where lesions similar to the primary inoculation site appear all over the body. The virus multiplies in epidermal cells after spreading hematogenously. Full recovery is expected without treatment.

Autoinoculation or inoculation of another person from the inoculation site is possible. The nose and the eyelid are the most common sites of inadvertent spread from the vaccination site. Permanent corneal damage is possible. Secondary transmission

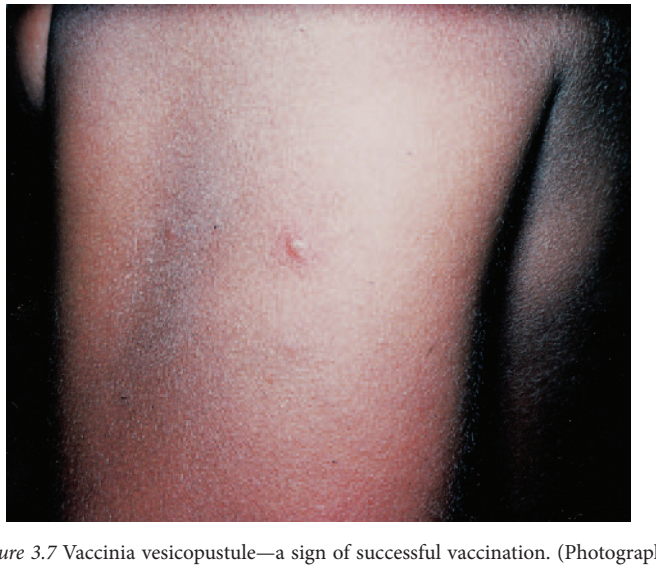

Figure 3.7 Vaccinia vesicopustule—a sign of successful vaccination. (Photograph courtesy of Harvey Blank, M.D., Department of Dermatology, University of Miami School of Medicine, Miami, FL.)

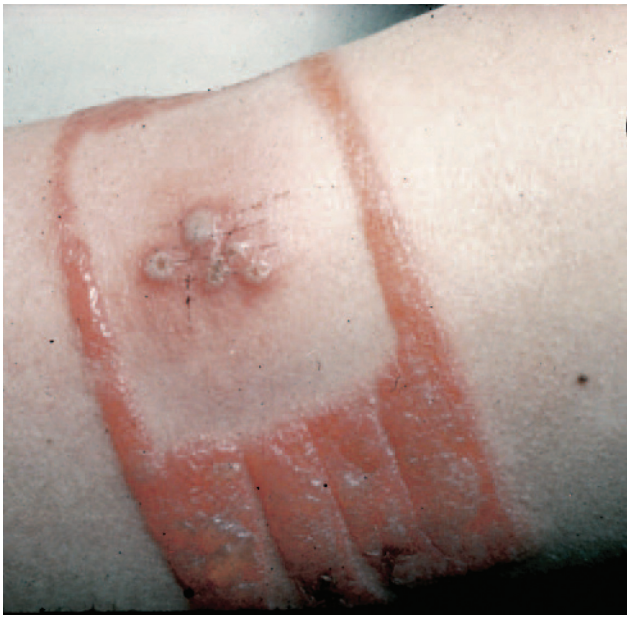

Figure 3.8 Vaccination site with contact dermatitis from tape.

may be increased in patients with other skin conditions, such as scabies, burns, impetigo, seborrheic dermatitis, pemphigus foliaceus, acne, and abrasions [40]. As the vaccinia virus is not attenuated, it can cause serious complications in some patients. Thymic aplasia, thymic dysplasia, acquired immune defects, and other impaired cell-mediated immunities contribute to progressive vaccinia, which is a very serious disease.

Adverse reactions involving the skin and the central nervous system (CNS) do occur. Increased mortality rates are reported in immunocompromised patients. Eczema vaccinatum, or extensive lesions in eczematous patients or eczematous family contacts of vaccines, is an occasional but serious problem. Complications such as eczema vaccinatum and vaccinia necrosum demonstrate a 10% and nearly 100% mortality rate, respectively, without treatment. Fortunately, mortality rates have decreased dramatically with the availability of vaccinia immune globulin (VIG) [18].

Nervous system complications are not responsive to VIG. Encephalopathy is most common in children between 6 months and 2 years of age and carries a mortality rate of 15–25%. An additional 25% are left with permanent neurological sequelae [36]. A demyelinating process has been described in adults. Adverse risks to the CNS may vary depending on the strain of the vaccinia virus. Cardiovascular complications such as ischemic heart disease, dilated cardiomyopathy, myocarditis, and pericarditis can occur in those with underlying heart disease [41]. Research findings reported in 2007 identified eight common genetic variations linked to fever susceptibility following smallpox vaccination. The study raises the possibility of identifying patient populations susceptible to more serious complications [42].

Dermatologists will be on the front line in the case of a smallpox outbreak. A study published in 2006 assessed dermatologist's knowledge of smallpox vaccination. Although most identified some contraindications to vaccination, such as immunosuppression and eczema, few identified myocardial infarction, angina, congestive heart failure, use of steroid eye drops, and non-emergency vaccination of patients under the age of 18 [43].

Table 3.5 Clinical Manifestations of Vaccinia

Time after exposure	Clinical manifestations	Laboratory analyses	Other notes
2–3 days	Papule appears ↓ (loculated and umbilicated) jennerian vesicle ↓	Laboratory analysis is usually not indicated since the source of infection is known	Lesion formation confirms successful vaccination Generalized vaccinia can occur at 6–9 days. Reaction may be more severe (see Figs. 3.9, 3.10, 3.11)
10 days	Pustule with surrounding erythema and induration (Fig. 3.7) ↓		Inoculation into eczema may occur. Infants and the elderly are at risk of spread, as are those with immune deficits (see Figs. 3.13, 3.14, 3.15)
12–13 days	Maximum erythema; lymphadenopathy; fever and malaise ↓		
22–24 days	Scab falls off		Pitted scar remains as evidence of vaccination

Vaccinees should be instructed to avoid high-risk individuals, including young children, pregnant women, immunocompromised individuals, and those with eczema, for 10–14 days after vaccination. Occlusive dressings and careful disposal of bandages can help prevent exposure to others [40]. Since the US military reinstated a program for smallpox vaccination in 2002, increased reports of cutaneous and systemic reactions in vaccinees and close contacts have been reported. In May 2007, a case of vulvar vaccinia was reported after intimate contact with a recently vaccinated member of the military. According to the US Department of Defense, 61 cases of contact vaccinia have been reported from 2002 to 2007 [44]. The CDC reports the risk of serious side effects from vaccination to be 1 in 1000. Life-threatening reactions such as eczema vaccinatum, progressive vaccinia, and post-vaccinal encephalitis occur in 14–52 in 1,000,000 vaccinees, with 1–2 in 1,000,000 dying as a result [43].

At this time, exposure is uncommon due to limited vaccination. However, with the threat of bioterrorism, large-scale vaccination is a possible future scenario. Hospitals may provide a unique environment for transmissibility and increased safety concerns. Large numbers of hospital employees would be vaccinated for the first time in an area concentrated with immunocompromised patients [40].

Future Applications

The vaccinia virus genome is large and can accept as much as 25 kb of foreign DNA, allowing it to be used to treat other disease processes as a live recombinant vaccine [50]. Its broad host range, including humans, laboratory animals, and common tissue culture cells allows for many potential applications [47,48]. Because vaccinia virus replicates in the cytoplasm, problems with host cell DNA integration and nuclear transcription errors do not occur [35].

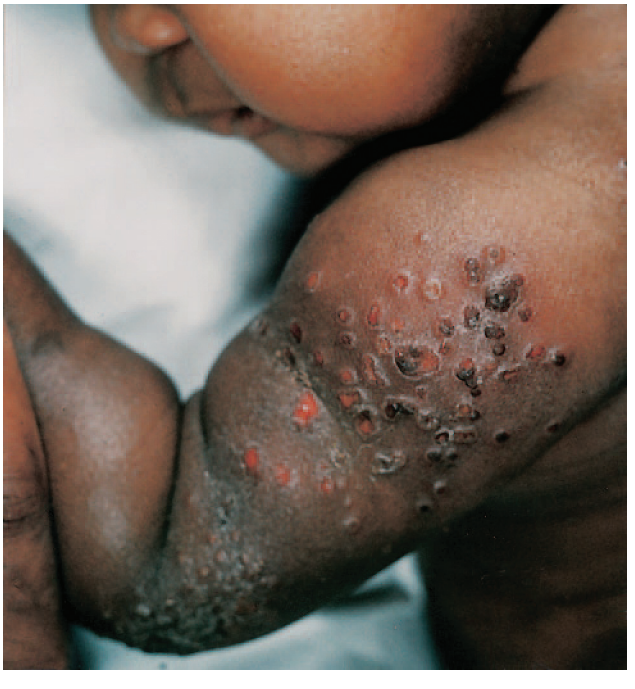

Figure 3.9 Local dissemination of vaccinia from vaccine site.

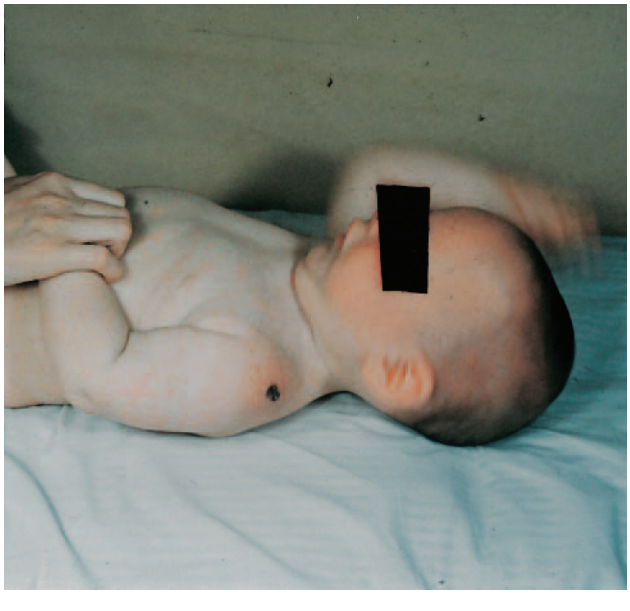

Figure 3.10 "Vaccinial roseola," a transient erythematous eruption following vaccination. (Photograph courtesy of Harvey Blank, M.D., Department of Dermatology, University of Miami School of Medicine, Miami, FL.)

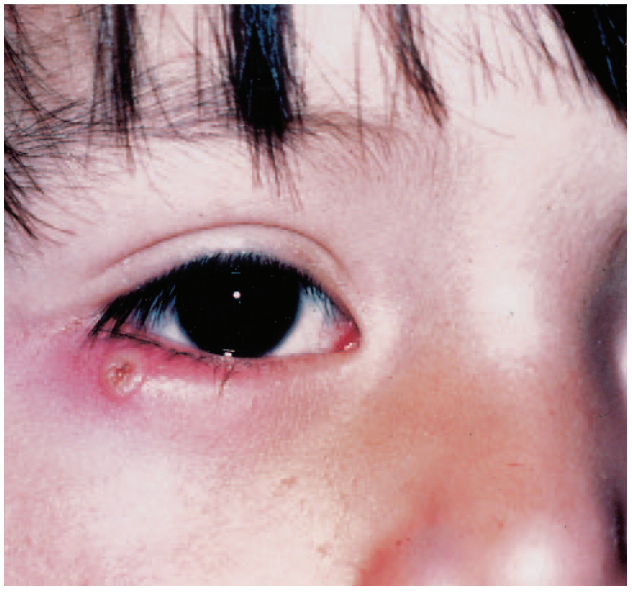

Figure 3.11 Autoinoculation of vaccinia to the lower eyelid produced a pustule. (Photograph courtesy of Roberto Arenas, M.D., Mexico City, Mexico.)

Recombinant vaccinia virus strains that express influenza hemagglutinin, hepatitis B surface antigen, and *Plasmodium falciparum* antigens have been produced [35]. An oral wild-life rabies vaccine has been developed [49]. The vaccinia virus is also currently under investigation for use as a HIV vaccine and for the treatment of various malignancies [50]. Potential laboratory applications include the insertion of virtually any coding sequence for a protein into the vaccinia virus genome, but its clinical use depends upon improving the safety of live vaccines and achieving high immune responses to recombinant protein [49,50].

Scientists are currently working on a recombinant interleukin-15 vaccine, which provides >1000 fold reduction in lethality of vaccinated athymic mice [51]. The vaccine also induces several-fold higher cellular and humoral immune responses that persist longer than that induced by the current vaccine. Recent research also supports using a higher dilution of the currently used vaccine. In 2007,

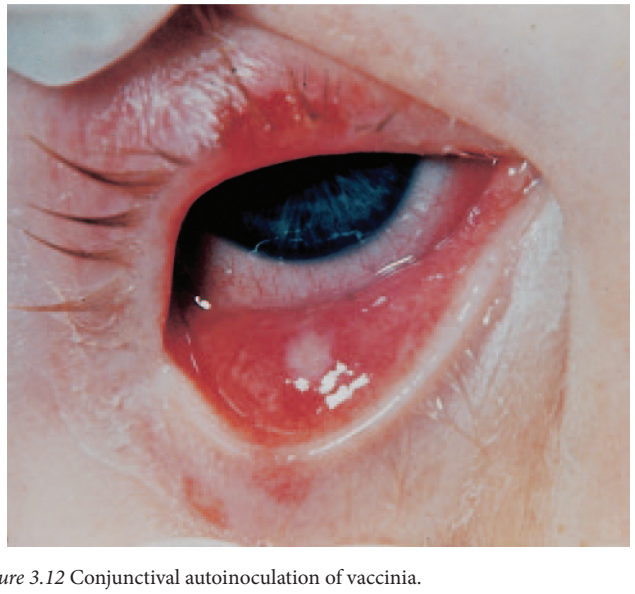

Figure 3.12 Conjunctival autoinoculation of vaccinia.

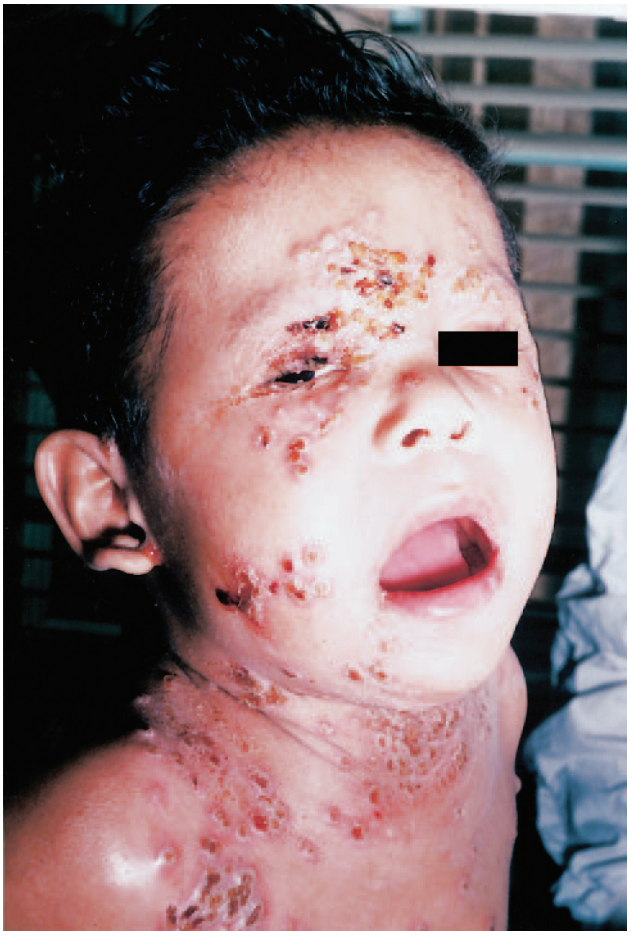

Figure 3.13 Eczema vaccinatum.

results demonstrated reduced morbidity without loss of effectiveness. Furthermore, in case of a smallpox outbreak, dilution would allow for production of many more doses of vaccine [52].

In the event of a terrorist attack, many subpopulations such as immunocompromised patients and pregnant women are contraindicated to receive vaccination. Cidofovir has been increasingly used as an antiviral drug in treating poxviruses. Historically, two drawbacks made this treatment difficult to use. Originally, cidofovir was only available in IV form. Also, renal side effects can be a problem with the large doses required to achieve appropriate intracellular concentrations. A new compound, which contains cidofovir in a partially degraded fat molecule, may prove viable as an oral treatment in the future [53].

Conclusion

The incidence of vaccinia decreased when routine smallpox vaccination with vaccinia was discontinued. In the future, the incidence of vaccinia may increase if the general public is vaccinated with potential exposure to our increasingly prevalent immunocompromised population.

Monkeypox

Definition

Monkeypox is an orthopox virus that occasionally infects humans. Monkeypox has been monitored closely in the post-smallpox eradication era.

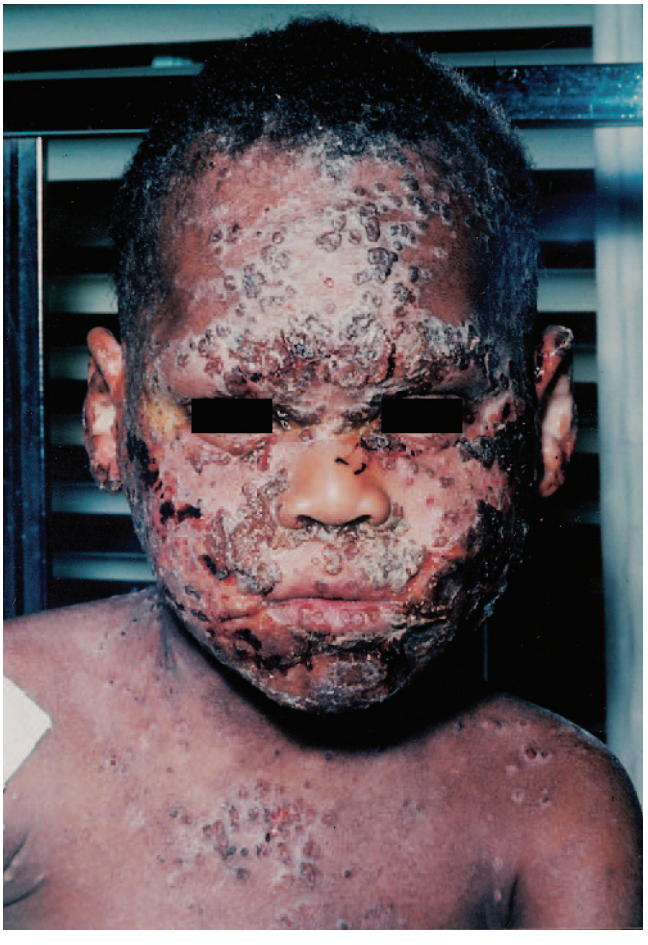

Figure 3.14 Eczema vaccinatum.

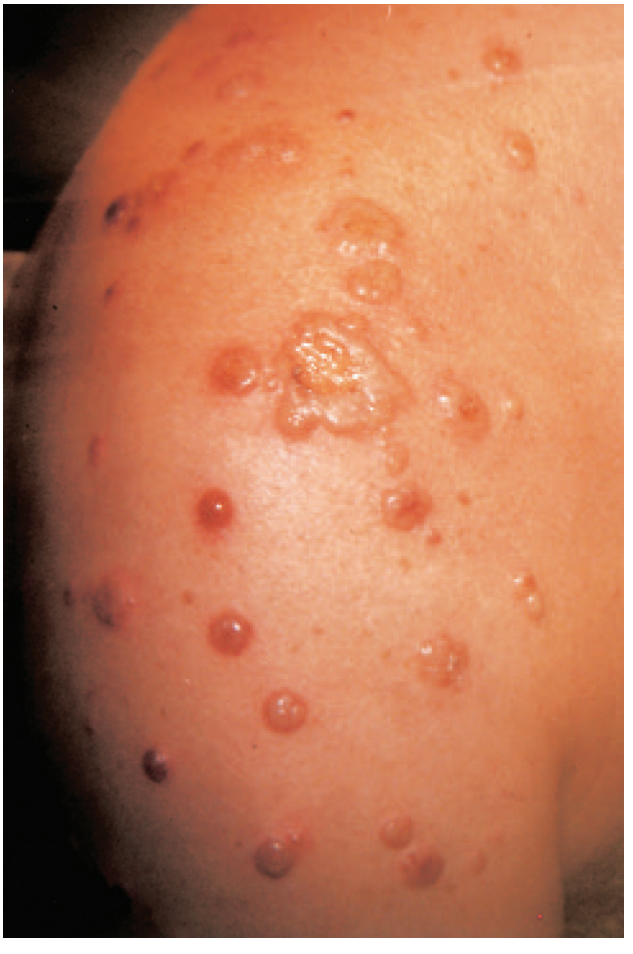

Figure 3.15 Early vesicopustules of eczema vaccinatum.

History

Monkeypox is the most serious orthopoxvirus infection in human beings since the eradication of smallpox in the 1970s. In 1958, monkeypox was discovered in laboratory monkeys [54]. Human monkeypox infection was identified in 1970. Residents and visitors of western and central Africa are most likely to be infected. Squirrels and monkeys in the rain forests of this area have been identified as reservoirs. However, rats, mice, and rabbits are also know to be infected with monkeypox [55].

Until 2003, monkeypox was isolated to the rain forests of central and western Africa. The first reports of monkeypox in the midwestern USA were in the spring of 2003, affecting people exposed to infected prairie dogs [56]. No fatalities were reported in the US outbreak. It is unknown if monkeypox has established an enzootic reservoir in the USA.

Pathogenesis/Epidemiology

Previous smallpox vaccination confers 85% protection against monkeypox [54]. Unvaccinated children are mostly affected, and deaths have been reported [4,8]. Preliminary DNA studies indicate only minor genetic variation among animal strains collected from 1970 through 1979 [57]. In 1986, the committee on orthopoxvirus infections identified human monkeypox as an insignificant worldwide health problem because of its low incidence in humans and their belief that inter-human transmission did not occur [12].

Surveillance reports from 1981 to 1986 documented 338 cases in the Democratic Republic of Congo (DRC; out of a 1982 estimated population of 5 million). In the 1996–1997 DRC outbreak, the attack rate was 22 cases per 1,000 population [58]. A review of 282 cases of monkeypox reported 50% of cases involved children aged 4 years or younger, and an additional 40% occurred in children from 4 to 14 years old [60]. The case fatality rate is generally 10% [61].

The first report of human monkeypox outside of Africa occurred in the USA in April 2003 [62]. Eight hundred small animals shipped from Ghana were implicated. An infected Gambian giant-pouched rat was housed with native prairie dogs in Illinois. The prairie dogs were subsequently sold as pets. Other animals from the shipment, including dormice and rope squirrels, also tested positive for monkeypox virus [63]. Seventy-two cases of human monkeypox were identified in six midwestern states in 2003. The risk of symptomatic infection correlated with the time and intensity of animal exposure [56].

Monkeypox virus has two different forms of transmission. Primary transmission occurs after skinning, handling, or consuming the meat of wild monkeys. Acquisition of the virus is from

Table 3.6 Differential Diagnoses of Vaccinia

Smallpox: Similar to generalized vaccinia but history of exposure to vaccinia via research or vaccination would differentiate

small lesions on the skin or mucous membranes of the animal. Secondary transmission involves close contact with infected humans. Not much is known about human to human transmission. The report from the 1996–1997 outbreak in the DRC suggests an 8–15% risk of secondary transmission to human contacts. However, investigation into past outbreaks and the recent US outbreak suggests that the predominant route of transmission is animal to animal and animal to human [54]. Reports from the 2003 US outbreak investigated 40 healthcare workers who had at least one unprotected exposure to a patient infected with monkeypox [64]. No signs or symptoms of monkeypox virus were reported in any of the exposed employees.

Some clinical differences were apparent in the US patients infected with monkeypox. Overall, the US infections demonstrated a decreased total number of lesions, a less predictable disease course, and no scarring. The morphology, evolution, and absolute number of lesions were more variable than those identified in African populations infected with monkeypox. Appearance and number of lesions seemed to vary in US individuals, including members of the same family with similar exposures. Also, healing of lesions with hemorrhagic crusts was distinctive of US cases. The differences may be attributed to mode of transmission, strain virulence, and the prevalence of prior smallpox vaccinations [63]. The route of exposure may also play a role in disease presentation and severity. Patients who sustained an invasive bite or scratch from an infected prairie dog were more likely to have a shorter incubation period without a febrile prodrome. This group also experienced pronounced signs of systemic illness and were more likely to be hospitalized [62] (Fig. 3.16).

Clinical Manifestations

The incubation period ranges from 10–14 days [54]. Viral spread resembles that of smallpox. Monkeypox and variola virus are unique in their capacity to cause severe systemic disease accompanied by a generalized vesiculopustular rash [62]. A febrile illness including severe headache, pharyngitis, and productive cough is often followed by lymphadenopathy within 2–3 days [54]. Lymphadenopathy is common and was noted in 47% of patients in the US outbreak. The submental, submandibular, cervical, and inguinal nodes are often involved and can be a reliable clinical sign to differentiate monkeypox from smallpox and chickenpox.

Lesions usually develop 1–10 days after the febrile illness and occur in crops, which progress from macules to papules to vesicles and pustules. Umbilication and desquamation may follow. The face, trunk, extremities, and scalp are typically involved. Lesions may be seen on the palms and soles as well (Fig. 3.17 and Table 3.7). Resolution generally occurs within 2–4 weeks. Children may demonstrate a more severe course and require ICU care [65]. Those who have been vaccinated against smallpox may demonstrate a milder form of disease with non-specific erythematous papules that resemble an arthropod bite [54] (Table 3.8). Mortality rates of 1–10% are reported in Africa. Factors such as amount of exposure to the virus, host immune status, vaccination status, present complications, and overall baseline health contribute to the prognosis.

Complications

Complications reported from African outbreaks include deforming scars, secondary bacterial infection, bronchopneumonia, respiratory distress, keratitis, corneal ulceration, blindness, septicemia, and encephalitis [54,65].

Treatment/Prophylaxis

Prior smallpox vaccination confers 85% protection from monkeypox, leading the CDC to recommend smallpox vaccination to those exposed in the recent US outbreak [66]. Recommendation

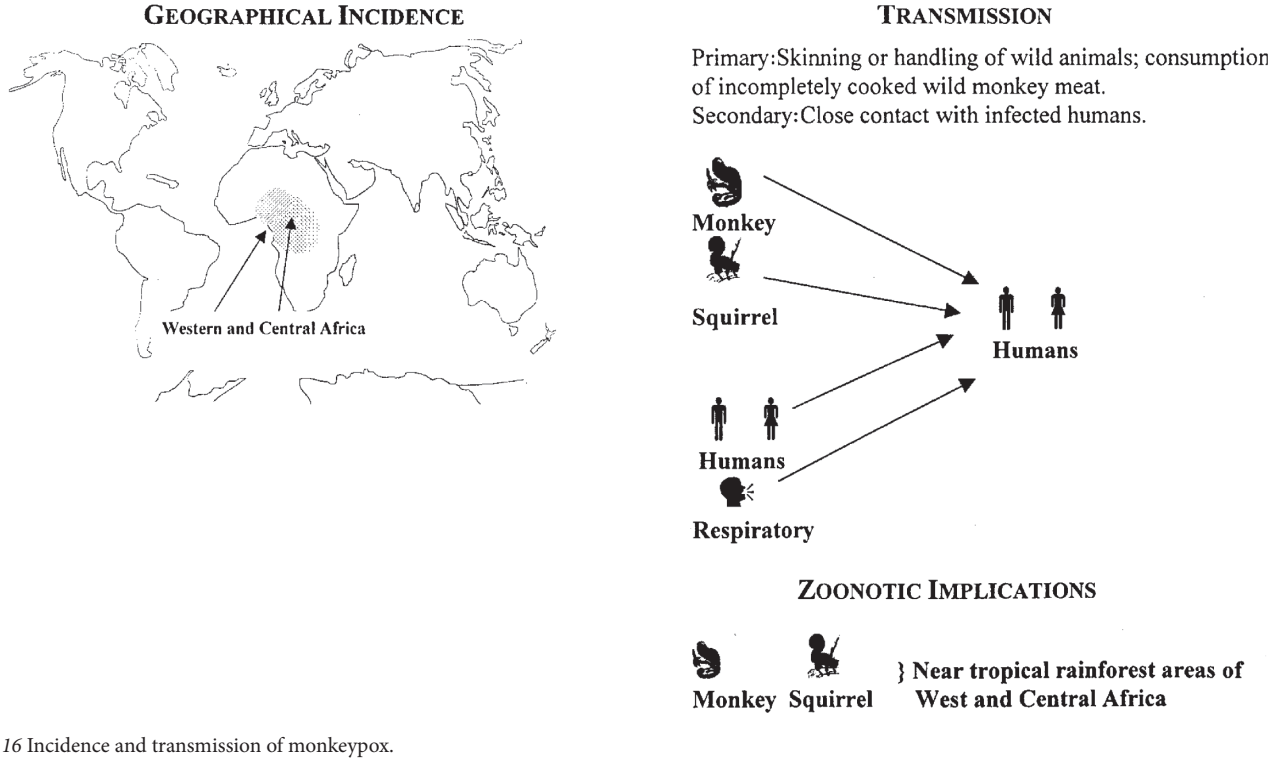

Figure 3.16 Incidence and transmission of monkeypox.

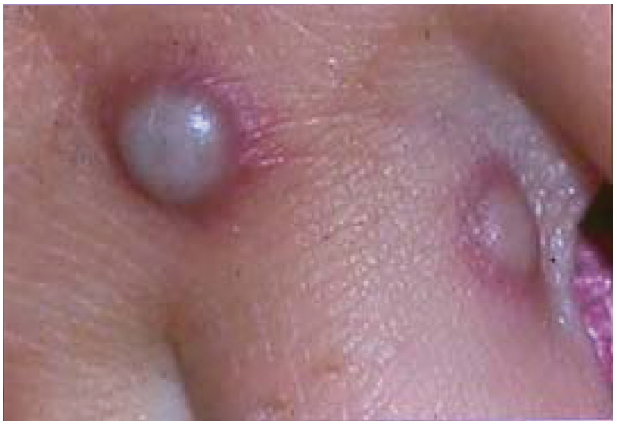

Figure 3.17 Monkey pox: Crop of pustules on fingers (Photograph courtesy of Dr. John Melski, Marshfield Clinic, Marshfield, WI).

of vaccination within 4 days of exposure is ideal, with some possible benefit up to two weeks after exposure. VIG is not efficacious for the treatment of monkeypox. In severe cases, cidofovir may be a treatment option.

Conclusion
Monkeypox produces a clinical disease in humans that is indistinguishable from smallpox, except for the more pronounced enlargement of cervical and sometimes inguinal lymph nodes, and a tendency for lesions to occur in crops. The incidence of this viral disease is increasing in frequency. Those without smallpox vaccination have increased susceptibility to monkeypox and increased severity of the illness.

Cowpox
Definition
An orthopox virus that infects cats, cows, rodents, and occasionally humans.

History
Cowpox is thought to be the original isolate used by Edward Jenner in the 18th century for development of his vaccine. This signaled the beginning of the age of vaccination [67].

Incidence
Cowpox has never been reported in the USA. Cowpox now appears to occur primarily in Europe and the former USSR [32,68]. Despite its name, cows are not the reservoir for infection. Infection in humans is primarily due to exposure to infected cats. Outbreaks in cattle are actually rare and of unknown origin. Infection in humans is relatively rare, but can be severe. Fewer than 150 cases of human cowpox have been reported. Exposure is more common in late summer and fall [69] (Fig. 3.18).

Pathogenesis
Cowpox virus is transmitted to humans primarily through contact with infected cats [69,70]. Typically, a broken area of skin, such as a minor abrasion, comes into contact with ulcers or lesions on an infected animal. Case reports have also noted contact through mucosa of the eye or nose. The natural reservoir of cowpox virus is believed to be small woodland animals, such as bank voles, wood mice, and short-tailed field voles [71]. It is important to note that this zoonosis is rarely contracted directly from a primary natural reservoir such as rodents, and not only accidental hosts commonly thought of such as cats. Although this is the exception rather than the rule, it became clearly evident after the report of a 14-year-old girl who contracted cowpox after caring for a sick wild rat [72].

Clinical Manifestations
After a week-long incubation period, infected humans develop a painless papule or papules at the site of inoculation that quickly evolve to a vesicular phase. Umbilicated pustules, which may become hemorrhagic, soon develop with surrounding erythema and edema. Transformation into a crust, eschar, or ulcer is often observed (Figs. 3.19 and 3.20). Lymphadenopathy is common, and fever or influenza-like illness may occur. Severe, but rarely fatal infections have been reported in atopic patients. In one fatal case, an 18-year-old eczematous patient on corticosteroids for asthma developed a smallpox-like eruption [68,73].

Only six cases of severe generalized skin infection have been reported, with atopic dermatitis as the main risk factor. The hands

Table 3.7 Clinical Manifestations of Monkeypox

Time after exposure	Clinical manifestations	Laboratory analyses	Other notes
1 day	Local inflammation	Isolation of virus from vesicular fluid or scabs	Transmission from animals was the most common form until 1996, when human-to-human transmission became more prominent
2–10 days	↓		
	Febrile response, with lymphadenopathy lasting 1–3 days, particularly in submandibular, cervical, and inguinal locations	(PCR) or hemagglutination	Infection is most common in children and the unvaccinated
	↓		Mucous membrane involvement is common
11 days	Severe headache, backache, and malaise		Monkeypox is a milder illness in those who received the vaccinia vaccine for smallpox
	↓		
	Papule		Severe generalized infection has approximately 10% mortality
	↓		
	Vesicle		
	↓		
Up to 4 weeks	Pustule		
	↓		
	Crust		