Verruca Pathology

Learning Objectives.

At the end of this course, you should be able to :

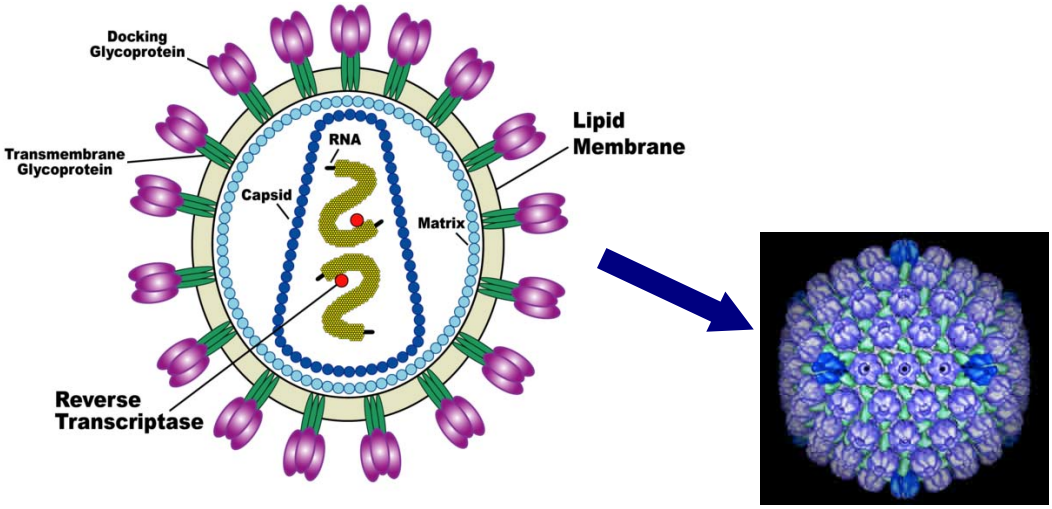
1. describe the method by which viruses enter a cell, replicate, and release further viral particles
2. understand the structure if the papilloma virus
3. **appreciate the role of the human papilloma virus in the development of cutaneous pathology**

Introduction

Viruses differ from all other infectious organisms in their structure and biology, particularly in their reproduction. Although viruses carry conventional genetic information in their DNA or RNA, they lack the synthetic machinery necessary for this information to be processed into new virus material. Viruses are metabolically inert and can replicate only after infecting a host cell and parasitising the host's ability to transcribe and/or translate genetic information. Viruses infect every form of life. They cause some of the most common and many of the most serious diseases of humans. Some insert their genetic material into the human genome and can cause cancer. Others have the ability to remain latent in different cell types and then reactivate at any time but especially if the body is stressed. Viruses are difficult targets for antiviral agents as it is difficult to target only those cells infected by the virus. However, many can be controlled by vaccines.

Viruses range from very small (poliovirus, at 30 nm) to quite large (vaccinia virus, at 400 nm, is as big as small bacteria). Their organization varies considerably between the different groups, but there are some general characteristics common to all:

- The genetic material, in the form of single-stranded (ss) or double-stranded (ds), linear or circular RNA or DNA, is contained within a coat or capsid, made up of a number of individual protein molecules (capsomeres)
- The complete unit of nucleic acid and capsid is called the 'nucleocapsid', and often has a distinctive symmetry depending upon the ways in which the individual capsomeres are assembled. Symmetry can be icosahedral, helical or complex
- In many cases, the entire 'virus particle' or 'virion' consists only of a nucleocapsid. In others, the virion consists of the nucleocapsid surrounded by an outer envelope or membrane. This is generally a lipid bilayer of host cell origin, into which virus proteins and glycoproteins are inserted.



The outer surface of the virus particle is the part that first makes contact with the membrane of the host cell. The structure and properties of the outer surface of the virus particle are therefore of vital importance in understanding the process of infection. In general, naked (envelope-free) viruses are resistant and survive well in the outside world; they may also be bile-resistant, allowing infection through the gastrointestinal tract. Enveloped viruses are more susceptible to environmental factors such as

drying, gastric acidity and bile. These differences in susceptibility influence the ways in which these viruses can be transmitted.

Infection of Host Cells

Virus particles enter the body of the host in many ways. The most common forms of virus transmission are:

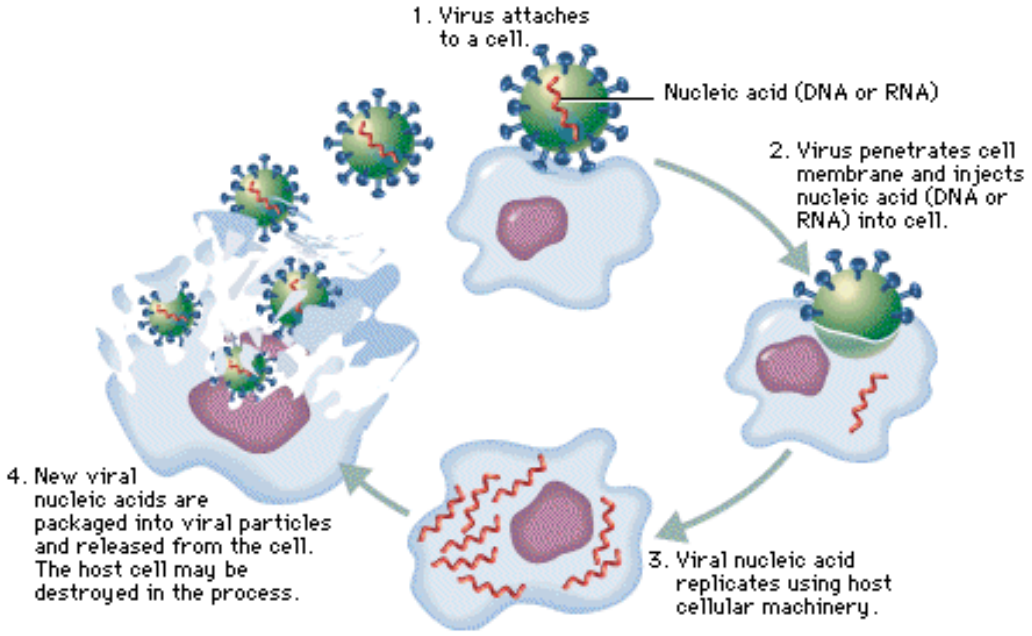
- via inhaled droplets (e.g. rhinovirus, influenza)
- in food or water (e.g. hepatitis A, noroviruses)
- by direct transfer from other infected hosts (e.g. HIV, hepatitis B)
- from bites of vector arthropods (e.g. yellow fever, West Nile virus).

Viruses show host specificity and usually infect only one, or a restricted range, of host species. The initial basis of specificity is the ability of the virus particle to attach to the host cell.

The process of attachment to, or adsorption by, a host cell depends on general intermolecular forces, then on more specific interactions between the molecules of the nucleocapsid (in naked viruses) or the virus membrane (in enveloped viruses) and the molecules of the host cell membrane. In many cases, there is a specific interaction with a particular host molecule, which therefore acts as a receptor. Attachment to the receptor is followed by entry into the host cell.

After fusion of viral and host membranes, or uptake into a phagosome, the virus particle is carried into the cytoplasm across the plasma membrane. At this stage, the envelope and/or the capsid are shed and the viral nucleic acid released. The virus is now no longer infective: this 'eclipse phase' persists until new complete virus particles reform after replication. The way in which replication occurs is determined by the nature of the nucleic acid concerned.

Replication



Viruses contain either DNA or RNA, never both. The nucleic acids are present as single or double strands in a linear (DNA or RNA) or circular (DNA) form. The viral genome may be carried on a single molecule of nucleic acid or on several molecules. With these options, it is not surprising that the process of replication in the host cell is also diverse. In viruses containing DNA, mRNA can be formed using the host's own RNA polymerase to transcribe directly from the viral DNA. The RNA of viruses cannot be transcribed in this way, as host polymerases do not work from RNA. If transcription is necessary, the virus must provide its own polymerases. These may be carried in the nucleocapsid or may be synthesised after infection.

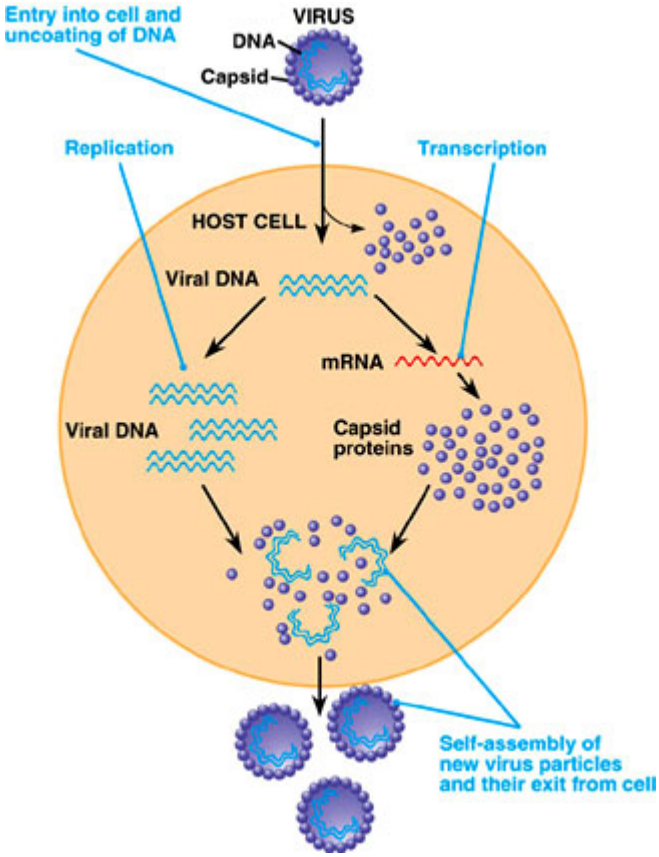
Once viral mRNA has been formed, it is translated using host ribosomes to synthesise viral proteins. Viral mRNA can displace host mRNA from ribosomes so that viral products are synthesised preferentially. In the early phase, the proteins produced (enzymes, regulatory molecules) are those that will allow subsequent replication of viral nucleic acids; in the later phase, the proteins necessary for capsid formation are produced.

In viruses where the genome is a single nucleic acid molecule, translation produces a large multifunctional protein, a polyprotein, which is then split enzymatically to produce a number of distinct proteins. In viruses where the genome is distributed over a number of molecules, several mRNAs are produced, each being translated into separate proteins. After translation, the proteins may be glycosylated, again using host enzymes.

Viral DNA may become complexed with host histones to produce stable structures. With herpes viruses, mRNA translated in the cytoplasm produces a DNA polymerase that is necessary for the synthesis of new viral DNA; adenoviruses use both viral and host enzymes for this purpose. With retroviruses (e.g. HIV), synthesis of new viral RNA occurs in the nucleus, host RNA polymerase transcribing from the viral DNA that has become integrated into the host genome.

The final stage of replication is assembly and release of new virus particles. Assembly of virus particles involves the association of replicated nucleic acid with newly synthesised capsomeres to form a new nucleocapsid. This may take place in the cytoplasm or in the nucleus of the host cell. Enveloped viruses go through a further stage before release. Envelope proteins and glycoproteins, translated from viral mRNA, are inserted into areas of the host cell membrane (usually the plasma membrane). The progeny nucleocapsids associate specifically with the membrane in these areas, via the glycoproteins, and bud through it. The new virus acquires the host cell membrane plus viral molecules as an outer envelope, and viral enzymes may assist in this process. Host enzymes (e.g. cellular proteases) may cleave the initial large envelope proteins, a process that is necessary if the progeny viruses are to be fully infectious. Release of enveloped viruses can occur without causing cell death so that infected cells continue to shed virus particles for long periods.

Insertion of viral molecules into the host cell membrane results in the host cell becoming antigenically different. Expression of viral antigens in this way is a major factor in the development of antiviral immune responses.


Entry into cell and uncoating of DNA
VIRUS
DNA
Capsid
Replication
Transcription
HOST CELL
Viral DNA
mRNA
Viral DNA
Capsid proteins
Self-assembly of new virus particles and their exit from cell

Key Learning Points :

1. Viruses have RNA or DNA but are absolutely dependent upon the host to process their genetic information into new virus particles
2. The outer surface of a virus (capsid or envelope) is essential for host cell contact and entry, and determines the capacity to survive in the outside world
3. Viruses are most often transmitted in droplets, in food and water or by intimate contact
4. Replication of viral RNA or DNA is a complex process, making use of host and/or viral enzymes
5. RNA of retroviruses becomes integrated into the host genome
6. New virus particles are released by cell lysis or by budding through the host cell membrane
7. Some viruses, such as herpes viruses, may become latent and require a trigger to resume replication; others replicate at a slow rate, persisting as a source of infection in symptomless carriers
8. A number of viruses transform the host cell by interfering with normal cellular regulation, resulting in the development of a cancer cell. This may be the result of the activity of viral or cellular oncogenes.

Human Papilloma Virus

There are over 120 different types of papillomavirus which can infect humans. Papillomaviruses are 55nm diameter, icosahedral, double-stranded DNA viruses and cause skin papillomas (warts). The different types that can infect humans show over 50% cross-hybridization of DNA, although not all types are common. Human papillomaviruses (HPV) are species-specific and distinct from animal papillomaviruses. They are highly adapted to human skin and mucosa and are ancient associates of our species; therefore, for most of the time they cause little or no disease. They show some adaptation to definite sites on the body:

- At least 40 types, including HPV 6, 11, 16 and 18, can infect the anogenital tract and other mucosal areas and are sexually transmitted
- HPV 1 and 4 tend to cause plantar warts
- HPV 2, 3 and 10 cause warts on the knees and fingers.

Papillomaviruses are generally transmitted by direct contact, but they are stable and can also be spread indirectly. For instance, plantar warts can be acquired from contaminated floors or from the non-slip surfaces at the edges of swimming pools, and in a given individual warts can be spread from one site to another by shaving.

After entering the body via surface abrasions, the virus infects cells in the basal layers of the skin or mucosa. There is no spread to deeper tissues. Virus replication is slow and is critically dependent upon the differentiation of host cells. Viral DNA is present in basal cells, but viral antigen and infectious virus are produced only when the cells begin to become squamified and keratinised as they approach the surface. The infected cells are stimulated to divide and finally, 1-6 months after initial infection, the mass of infected cells protrudes from the body surface to form a visible papilloma or wart. There is marked proliferation of prickle cells, and vacuolated cells are present in the more superficial layers.

Immune responses eventually bring virus replication under control and, several months after infection, the wart regresses, although this can take up to two years. It seems likely that viral DNA remains in a latent state in the basal cell layer, infecting an occasional stem cell, and is therefore retained within the layer as epidermal cells differentiate and are shed from the surface. Hence, when patients are subsequently immunocompromised (e.g. post-transplant) crops of warts may result from reactivation of latent virus in the skin.

HPVs establish their productive life cycle exclusively in stratified epithelium of skin or mucosa. These tissues are complex, composed of several different cell types, majority of which comprise layered sheets of keratinocytes in various stages of differentiation. The remaining cell types include melanocytes, Langerhans cells and Merkel cells.

There are two types of dividing keratinocytes in the epidermis:

- slowly cycling undifferentiated stem cells
- cells capable of transient proliferation in basal cell compartment

These undifferentiated proliferating keratinocytes are the initial target for productive HPV infections. Some of the infected cells loose the contact with the basal membrane and move up into the suprabasal compartment of proliferating cells, where they establish latently infected proliferating cell population. Subsequent steps in the viral life cycle are strictly dependent on the differentiation of the host epithelium. During the normal skin development, uninfected keratinocytes exit the cell cycle and commit to the terminal differentiation ultimately leading to apoptosis. HPVs have evolved mechanisms to adapt to the normal cellular growth control pathways and adjust their DNA replication cycle and maintenance cycle to contend with all different cell states. The successful infection of a keratinocyte triggers the initial amplification of papillomaviral (PV) DNA copying. This is followed by the stable maintenance phase of the HPV genome per cell. Finally, vegetative amplification of the viral DNA is

performed. The precise mechanisms responsible for the switch from one replication mode to another is unknown, however, it seems to be closely bound up with the differentiation state of the cells.

The second amplification round in a subset of spinous cells results in the synthesis of viral capsid proteins and assembly of virus particles that are ultimately shed from a small number of superficial cells during desquamation. The HPV replication cycle takes at least 3 weeks, as this is the time required for the keratinocyte to undergo complete differentiation cycle.

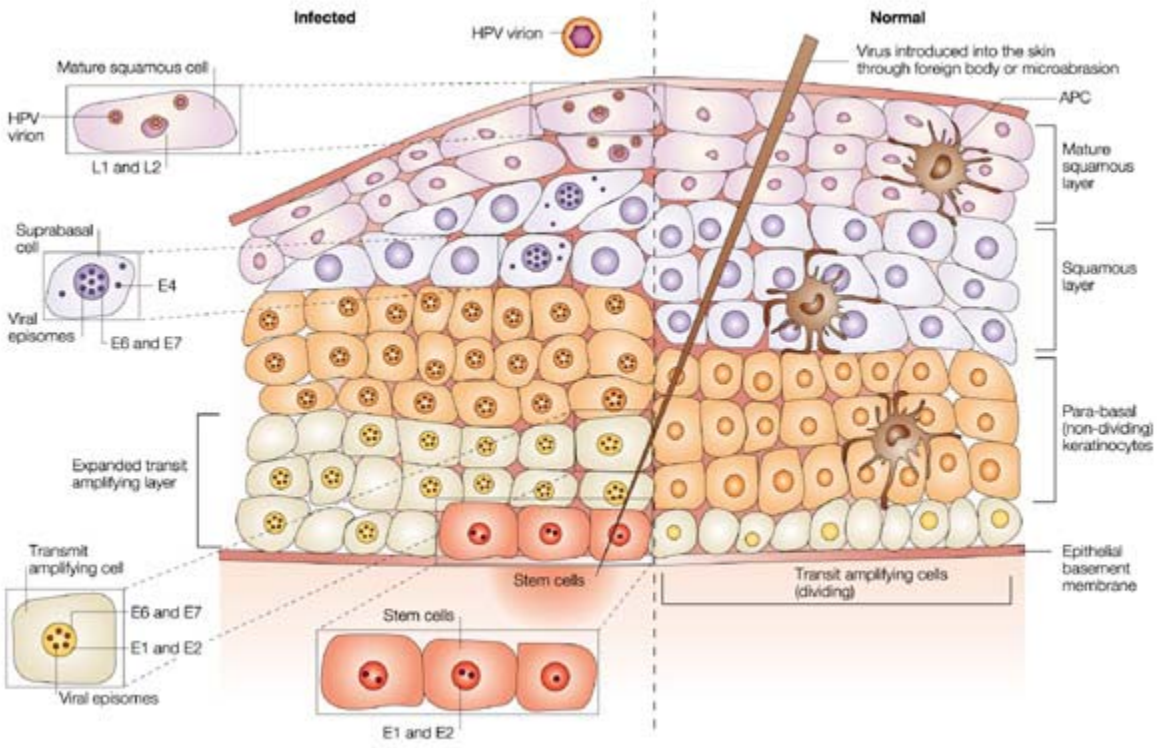
E6/E7 proteins

The viral oncogenes (oncogenes are mutated genes which make cells proliferate), E6 and E7, are thought to modify the cell cycle so as to keep the differentiating host keratinocyte in a state that allows amplification of viral genome replication and consequent late gene expression. E6, in association with host E6 AP (associated protein), acts to modify p53 leading to its proteosomal degradation. Normally, p53 is a protein that functions to block the cell cycle if the DNA is damaged. If the damage is severe this protein can cause apoptosis. p53 levels are increased in damaged cells. This allows time to repair DNA by blocking the cell cycle. A p53 mutation is the most frequent mutation leading to cancer.

E7 (in oncogenic HPVs) acts as the primary transforming protein. E7 competes for retinoblastoma protein (pRb) binding, freeing the transcription factor E2F to transactivate its targets, thus pushing the cell cycle forwards.

All HPV can induce transient proliferation, but only HPV 16 and 18 can immortalise cell, i.e. stop the normal limitation of cell division, allowing infinite cell divisions. In the upper layers of the host epithelium, the late genes L1 and L2 are transcribed/translated and serve as structural proteins which encapsidate the amplified viral genomes. Encapsidation is the process of incorporating a nucleic acid sequence (e.g., a vector, or a viral genome)

into a viral particle. Once the genome is encapsidated, the capsid appears to undergo redox-dependent assembly/maturation which is tied to a natural redox gradient that spans both suprabasal and cornified epithelial tissue layers. Redox refers to a reduction-oxidation process. This stabilises virions, and increases their specific infectivity. Virions can then be sloughed off in the dead squames of the host epithelium and the viral lifecycle continues.



Daughter cells of epithelial stem cells divide along the basement membrane and then mature vertically through the epithelium without further division (right side of diagram). After introduction of HPV into stem cells in the basal layer of the epithelium, expression of viral non-structural proteins occurs (left side of diagram). Under the regulation of these proteins, the dividing-cell population expands vertically and epithelial cell differentiation is delayed and is less complete. Viral proteins are expressed sequentially with differentiation as shown, and mature virions are produced only in the most superficial layers of the epithelium. Intraepithelial antigen-presenting cells (APCs) are depleted in the HPV-infected epithelium.

Latency period

Once an HPV viron invades a cell, an active infection occurs, and the virus can be transmitted. Several months to years may elapse before squamous intraepithelial lesions (SIL) develop and can be clinically detected. The time from active infection to clinically detectable disease makes it difficult for someone who has become infected to establish the source of infection.

Clinical presentation

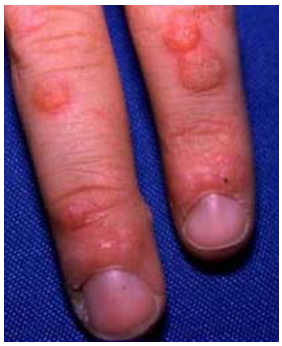
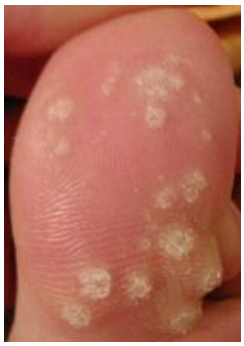
There are multiple presentations of HPV infection, but typically they present as warts. Cutaneously, there are a variety of shapes and types. Warts can be:

- filiform with finger-like projections
- flat topped
- flat because they grow inwards due to external pressure (e.g. plantar warts)
- a cauliflower-like protuberance (e.g. genital warts)
- a flat area of dysplasia, such as on the cervix.

Common warts (verruca vulgaris) —

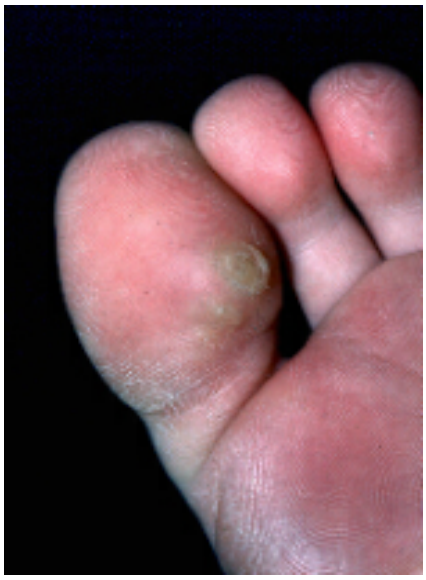
Common warts are well-demarcated, rough, hard nodules or plaques with an irregular surface. Common sites for cutaneous warts include the dorsum of the hand, between the fingers, periungually, on the palmer and plantar surfaces, and rarely, on mucous membranes. Common warts are usually asymptomatic unless located on a pressure point, and rarely undergo malignant degeneration. Common verruca represent the most frequent clinical lesions produced by the human papillomavirus. The morphology can vary considerably from relatively smooth, sessile lesions (lying close to the skin surface),to large pedunculated lesions. These are particularly common in childhood where immature immunologic resistance and frequent skin-to-skin contact with other children increases the likelihood of transmission. Another group who exhibit a high infection rate are meat, poultry and fish

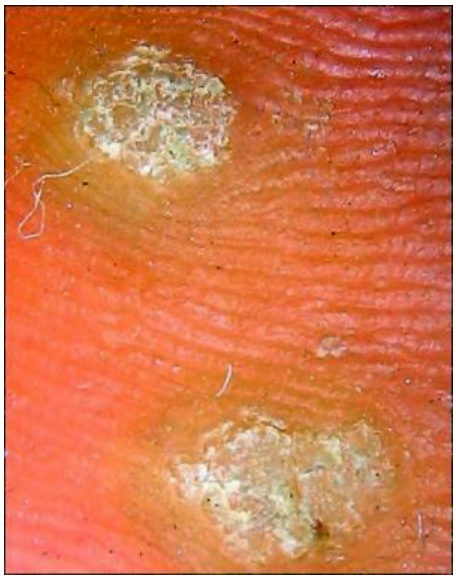
handlers. So called "butchers warts" are usually caused by HPV 2 or 7, although the reason why this occurs more commonly in this group of workers is unknown. Individuals with atopic dermatitis appear to have a mild T-cell defect as suggested by a higher prevalence of infection with common warts, and exhibit more numerous lesions. The presence of atopy (allergic predisposition) should be suspected when older children or adults present with more than a dozen common verruca and no other cause of immunosuppression.


Plantar warts (myrmecial) —

Plantar (or myrmecial) warts affect mostly adolescents and young adults. They present as thick painful endophytic plaques located on the plantar or palmar surfaces. The lesion is often covered by a thick callous, reduction of which reveals punctate bleeding. One of the distinguishing features is that plantar warts lack dermatoglyphics (the ridges, whorls, and loops that are characteristic of a palm, digit, or sole print of an individual), with the skin striae appearing to form around rather than through the lesion. These lesions are remarkable for their thickness due to their presence in the acral skin of the hands and feet. The greater depth of infected tissue makes these warts more difficult to treat successfully compared with warts in non-acral skin.

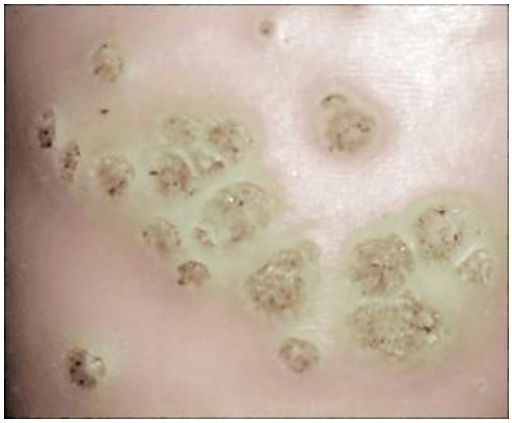


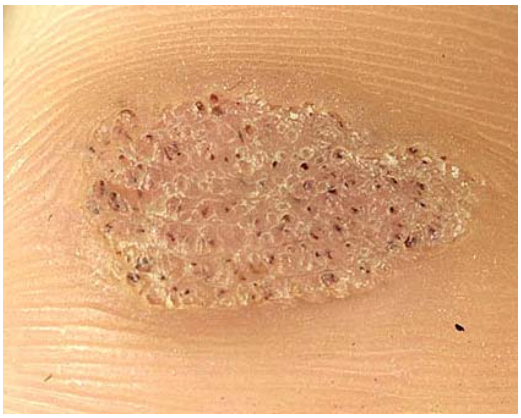


HPV 1 is the most common cause of palmar and plantar warts, although HPV 2 and other viruses cause them. HPV 60, a much less common cause of plantar warts, and is associated with palmoplantar warts that have cystic components. HPV 63, also an uncommon cause of plantar warts, and can cause a punctuate, mosaic-type plantar wart

Flat warts (mosaic warts) –

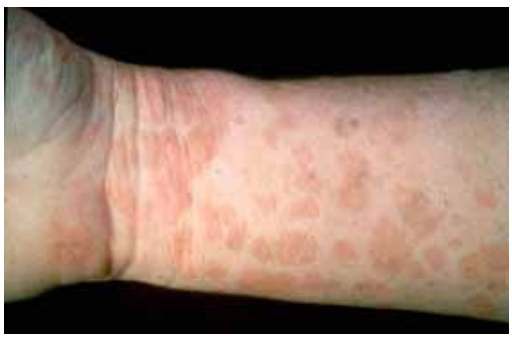
Warts of this type are less exophytic (out-growing) than common warts, frequently presenting as several or dozens of subtle papules 2-4 mm in diameter elevated above the surface by less than a millimeter or so. They can be quite subtle, and may be missed by a casual observer. Pigmentary disturbances may be the most disturbing part of a flat wart infection to the patient. These lesions are frequently a problem on the face and glabrous (hairless) skin of non-immunocompromised individuals. Flat warts in this setting are commonly caused by HPV 3, 10 and occasionally by HPV 2. A number of other HPV types cause flat warts in glabrous skin, but most of these are seen exclusively in immunocompromised individuals and in epidermodysplasia verruciformis. Treatment can be difficult even in an immunocompetent individual.

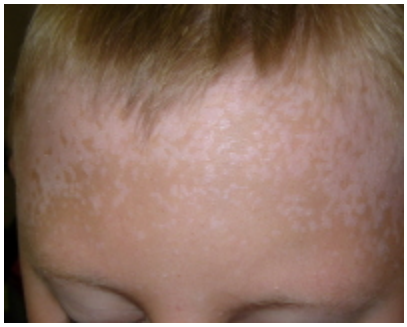




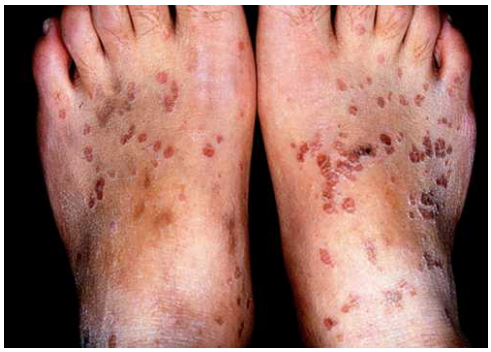
Epidermodysplasia Verruciformis -

Epidermodysplasia Verruciformis (EDV) is an autosomal recessive disorder of cutaneous immunity which makes affected individuals susceptible to a subset of warts not seen in other individuals. The advent of transplant technology and HIV / AIDS has changed this, and now cases of clinical infection with these viruses are seen in immunocompromised individuals. HPV types characteristic of the disorder include HPV 3, 5, 8, 9, 10, 12, 14, 17, 20, 21, 23, 25, 28, 38, 47, 49.





Warts in EDV are typically flat, numerous and subtle, but may be erythematous. When the disease begins to manifest in childhood, the warts sometimes give the clinical appearance of tinea versicolor. They can involve almost any area on the body, but tend to be more prominent on the extremities, especially the arms.



The combination of HPV infection, relative immunosuppression and sunlight are a potent carcinogenic combination; if these individuals are not recognized and treated appropriately, they are at substantial risk of developing skin cancers, such as squamous cell carcinoma (Bowen's disease).

Relative effectiveness of treatments

A 2006 study assessed the effects of different local treatments for cutaneous, non-genital warts in healthy people. The study reviewed 60 randomized clinical trials dating up to March 2005. The main findings were:

- overall there is a lack of evidence (many trials were excluded because of poor methodology and reporting).
- the average cure rate using a placebo was 27% after an average period of 15 weeks.
- the best treatments are those containing salicylic acid. They are clearly better than placebo.
- there is little clinical trial data for the absolute efficacy of cryotherapy
- two trials comparing salicylic acid and cryotherapy showed no significant difference in efficacy.
- one trial comparing cryotherapy and duct tape occlusion therapy showed no significant difference in efficacy.
- evidence for the efficacy of the remaining treatments was limited.

Key Learning Points :

1. HPV are species-specific, and are highly adapted to human skin and mucosa.
2. They are generally transmitted via direct contact, but may also transmit indirectly.
3. The virus will not affect tissues deeper than the basal layer of the epidermis.
4. Differentiation and proliferation of the host epithelium is affected by the virus.
5. HPV produces transient proliferation in keratinocytes.
6. Lesions may have many presentations, and can appear on any epidermal surface.
7. Patients who are immunosuppressed are at risk of developing multiple, resistant lesions, which are difficult to treat.