University of Jordan
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Virology

Number: 2

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Subject: Poxviridae

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In this lecture, we are going to talk about the following viruses:
- VZV (Varicella-zoster virus)
- HHV-8 (Human Herpes Virus 8)
- Pox viruses
- Human Papilloma virus (HPV)

-Dr. Ashraf was reading the slides during the lecture and nothing was explained well. In this sheet, I tried to clarify the slides. So, if you want to study what was mentioned in the lecture, go to slides.

Varicella-zoster Virus (VZV)

- It's called Varicella-zoster virus because it causes two diseases:
1- Varicella (Chickenpox) : جدري الماء
2- Zoster (Shingles) : الحزام الناري

- General properties:
    - They belong to the alphaherpesvirinae subfamily.
      - alphaherpesvirinae viruses are characterized by rapid rate of replication and neuronal latency. So, like all herpes viruses, it can become latent after the primary infection.

      - dsDNA → it replicates in the nucleus → during infection, new viruses are assembled in the nucleus and that's why inclusion bodies are formed in the nucleus rather than in the cytoplasm.

      - Enveloped

      - It represents one antigenic serotype that's distinct from HSV-1 and HSV-2 although they are similar to one another and there's cross-reactivity between them.

      - Incubation period : 11-21 days

      - Chickenpox occurs most frequently during winter and spring months.

      - The major route of transmission is the respiratory route. Also, direct contact with skin lesions may cause transmission.

Remember that the incubation period is the period between the entry of the offending agent (virus) and the appearance of symptoms and during which transmission of infection is possible. So, during the 11-21 days preceding the appearance of chickenpox rash, transmission is possible.

- Transmission is possible during the incubation period and the rash phase but when is it the greatest?
1-2 days before the rash onset and lasts 3-4 days in the rash phase.

Epidemiology:

- Never forget this:

Chickenpox is caused by primary infection, while shingles is caused by reactivation.

- Primary varicella is an endemic disease. Varicella is one of the classic diseases of childhood, with the highest prevalence occurring in the 4-10 years old age group.

- Varicella is highly communicable, with an attack rate of 90% in close contacts.

- Most people (90%) become infected before adulthood but 10% of young adults remain susceptible.

- Herpes zoster, in contrast, occurs sporadically and evenly throughout the year.

Pathogenesis:

- What proves that chickenpox and shingles are caused by the same virus?
We said that chickenpox is caused by primary infection, while shingles is caused by reactivation. If someone with shingles comes into contact with individuals that have never developed chickenpox, the virus will be transmitted causing primary infection that's manifested by chickenpox rash. So, they are caused by the same virus (VZV).

Poxviridae

General Characterstics .

- They infect humans, birds, mammals, and insects.

- It's brick-shaped or ovoid (look at the picture in the first slide). [Pox looks like a box.]
  - DNA virus
  - Its DNA is double-stranded
  - Enveloped
  - It multiplies in the cytoplasm (Remember: All DNA viruses replicate in the nucleus except Pox viruses).

- DNA viruses are more dependent on the host cell. Why ?

Our nuclear DNA is continuously replicated whenever a cell wants to divide, so our cells are equipped with every single enzyme needed for DNA replication or for transcription. This is the reason why DNA viruses take it as an advantage and go into the nucleus and replicate there. On the other hand, there's no mechanism for RNA replication or transcription in our normal cells, that's why RNA viruses find nothing to depend on in our cells and have to bring the equipments of replication and transcription with them.

Now, knowing that poxviruses are the largest DNA viruses, do you predict that they will replicate in the nucleus or in the cytoplasm? And do you predict that they encode for replication and transcription enzymes or not?

- Poxviruses replicate in the cytoplasm and encode all essential enzymes, proteins, and factors needed for viral replication.

- Their capsids are neither helical nor icosahedral (lacks normal capsid). Instead, they have layers of lipoprotiens and fibrils on surface

- Variola (small pox), Vaccinia, Moluscum contagiosum, orf, cowpox, and pseudocowpox

Smallpox

"Smallpox has been placed in a box and buried."
- Smallpox was completely eradicated globally in 1977. So why are we studying such a disease?
Because poxvirus is a potential warfare agent and can be used in bioterrorism. Therefore, knowledge and understanding of smallpox pathogenesis and disease is important for any future control of outbreaks of poxviral diseases. Refer to page 393+394 in MRS, 6th edition. There's nice information there.

- A lot of viruses cause fatal diseases but only a few diseases are completely eradicated. Smallpox was eradicated completely in 1977 and this was accepted by the WHO in 1980. So, what makes the eradication of this disease possible?
1- There's no animal reservoir for this virus. Monkeypox for example is still present although it's similar to Variola because there's an animal reservoir for it. Shortly, animal reservoirs make infections uncontrollable
2- There's no asymptomatic carriers.

Variola (small pox)

- Smallpox is caused by either Variola major or Variola minor. Although these viruses are very similar to each other (indistinguishable antigenically), the mortality rates differ considerable and hence the names [major & minor].

- Mortality rates (Variola major → 3-40% , Variola minor → less than 1 %).

•Variola major (smallpox) (3-35%), V. minor (alastrim) (<1%).

•Uniform papulovesicular rash, pustules with significant mortality.

- Smallpox virus is highly contagious and can survive well in the extracellular environment. Infection can be transmitted through saliva droplets, contact with skin lesions and fomites.

Why are we concerned about the recurrence of smallpox despite its complete eradication? Because if it's used as warfare agent, it will cause a serious problem for the following reasons:
(1) smallpox is one of the most stable viruses; (2) it can remain stable for a long time, if freeze-dried;(3) it is unaffected by environmental conditions; (4) scab forms are stable for 1 year at room temperature and in one case it has been found to be stable for 13 years in a laboratory; (5) it has high infectivity among humans; (6) it is associated with high susceptibility among populations (routine vaccination against smallpox ended in 1972, and current vaccine supplies are limited); (7) there is a risk that healthcare providers may not promptly recognize and respond to early cases; and (8) there is an absence of specific antiviral treatment.

Pathogenesis:

How does the virus affect the host?

- The virus switches off cellular protein synthesis to synthesize its own proteins.
- The virus impairs the innate immunity and interferes with cell-mediated immunity [For further details, refer to Sherris Medical Microbiology, 6th edition, page 204].

- Changes in the cell permeability.

  - Cell lysis (cytolysis).

Manifestations and diagnosis

- The incubation period of smallpox is usually 12 to 14 days, although in occasional fulminating cases it can be as short as 4 to 5 days.

  - The typical onset is abrupt, with fever, chills, and myalgia, followed by a rash 3 to 4 days later.

- The rash evolves to firm papulovesicles that become pustular over 10 to 12 days, then crust and slowly heal.

- The main distinguishing difference between smallpox and chickenpox:
Chickenpox → Asynchronous lesions (skin lesions are at different stages)
Smallpox → Synchronous lesions (skin lesions are all at the same stage)

Other differences are mentioned in the following table:

Chickenpox	Smallpox
VZV (Herpesviridae)	Variola (Poxviridae)
Superficial lesions	Deep lesions
Prominent on the chest	Prominent on the face and palms
Palms and soles are not affected - the axilla is affected	- Palms and soles are affected - the axilla is not affected

- It's not logical that skin lesions can lead to death, hence, there must be certain complications of smallpox that cause death. These complications are:
1- pneumonia 2-bone infections … etc
OR Bacterial superinfection

- Sledgehammer smallpox : It's a form of smallpox in which the patient suffers from hemorrhagic rash. Massive bleeding occurs from skin lesions, as well as from the mouth and nose and other areas of the body.

- Sledgehammer smallpox is highly fatal and death occurs 5-7 days after infection.

- Diagnosis:
Read this → http://www.emedicinehealth.com/smallpox/page4_em.htm

- It's mainly by the clinical presentation of smallpox rash.

- Scraping of vesicles, culture, EM and PCR are used for diagnosis.

- **Molluscum Contagiosum**
- It's a benign, cutaneous disease caused by molluscum contagiosum virus which belongs to the poxviridae family.
- Transmission:
As the name implies, this disease is contagious, so it's transmitted by direct contact with the virus (touching the skin lesions of an infected person, touching a towel or anything with a virus on it, sexual contact … etc).

- Incubation period: 2-8 weeks

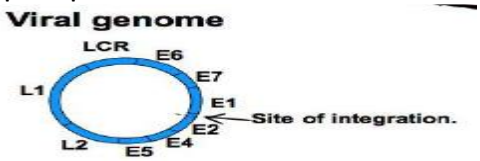
After the incubation period, symptoms will appear. This disease is characterized by skin lesions.
- Skin Lesions:
Nodules that are: painless, pearl-like, range from 2-10 mm in diameter, and umbilicated.
- Umbilicated = a lesion having dimpled center.

- This disease is self-limited, disappears in 2-12 months and causes no systemic symptoms. Therefore, treatment is optional.
- Almost all patients with molluscum contagiosum prefer to remove the lesions since they are cosmetically not acceptable.
- Treatment: Removal of nodules by curettage.
https://www.youtube.com/watch?v=qoNSjetgIcA

ORF & Milker nodules and cowpox
Refer to slide9 to see how they look like.

HPV

- Small, naked, double stranded circular DNA virus with an icosahedral capsid.
- The capsid is composed of 2 proteins L1(the major capsid protein) and L2 (the minor capsid protein).
- Its genome is double-stranded. It encodes eight early genes (E1-E8) and 2 structural capsid proteins L1 and L2.



- The virus replicates in the nucleus, so everything it encodes must be for regulation of viral replication and transcription (E1 &E2), and transformation (E7 &E6) not for the replication itself.

- The virus does not encode any polymerase and it's therefore dependent on the host cell transcription and replication machinery.

- There are over 100 different types of the HPV virus - most types are totally harmless.
- HPV causes papillomas and warts in a wide range of higher vertebrates.

- Great Genomic Diversity:
It's difficult to propagate HPV in cell culture, this leads to the following facts:

- Virological studies on HPV always fail. Therefore, HPV replication cycle is not very well understood.
- Most of what we know about HPV is attributable to the efforts made in molecular biology and gene expression studies.
- Due to the wide genomic diversity of HPVs, each genotype is designated a number.

HPV replication

Before we start, watch this video
https://www.youtube.com/watch?v=AZOnAuElJHk

- HPV replication is not well understood.
- Before going into HPV replication, purposefully, I will revise certain aspects of epithelial cell differentiation in the skin.
- Our skin is composed of 4-5 layers and its epithelium is of the stratified squamous type. As we go up from the basal layers to the apical layers, cells become more differentiated and more flattened. HPV replication steps depend on this sequence of differentiation.
- Now we will see how HPV enters cells, synthesizes proteins, replicates DNA and how it is released out of cells to infect other cells.

- HPV gains entry into our skin cells through abrasions or any cut in the skin to reach the basal layer. Then, the virus is internalized, uncoated and delivered into the nucleus. Now, basal layer cells become infected with HPV.

  - What HPV wants to do is to synthesize non-structural proteins when it's present in basal- and middle-layer cells and then synthesize structural proteins (L1 & L2) when it reaches the apical layer, preparing new virions to be released from the apical layer to infect other regions of the skin.

- Early stage protein synthesis starts with the synthesis of E6 and E7 which bind to p53 and RB, respectively. Consequently, two of the most important tumor suppressor proteins are nullified, initiating unlimited cell division and a mutator phenotype, paving the way to transformation.

  - As cells go up and become more differentiated, synthesis of E1 and E2 which regulate viral transcription and replication starts.

- Late protein synthesis starts in the apical-layer cells. This involves the synthesis of capsid structural proteins (L1&L2).

Now, how HPV DNA is replicated?
When HPV is in the basal layers, there's no need for high rate of DNA replication. DNA is just replicated to provide viral DNA for latent infection. On the other hand, when HPV is in the apical layer, high rate of DNA replication is required as the virus needs a lot of copies of DNA to be assembled into new virions that are prepared for release.

- Briefly, viral DNA synthesis occurs at two levels directed by cellular DNA polymerase
Lower epidermis: viral DNA for latent infection

Differentiated epithelial cells: vegetative DNA replication

- New viruses are assembled in the nucleus and released by cell lysis.

HPV prevalence

- It's the most common STD in the US.

Numbers mentioned here are different from those in slides. Dr. Ashraf will not ask us about numbers, so I will mention the correct numbers.

*14 millions are infected yearly
* 79 million Americans are already infected with HPV
* An estimated 9.2 million sexually active adolescents and young adults 15-24 years of age are infected with genital HPV

- An estimated 5%-30% of people infected with genital HPV are infected with multiple types of the virus
- 316,000 initial visits to physicians' offices -genital wart diagnosis

HPV causes cutaneous lesions, genital warts, and many types of cancer, each with its distinct HPV genotypes.
- In children and young adults, HPV causes cutaneous (nongenital) lesions. At older ages, specific immunity develops and the incidence of cutaneous warts becomes less.
- A single sexual exposure to an infected person may transmit the infection 60% of the time; usually the infected person is asymptomatic.

- Having multiple sex partners is the major risk factor for acquiring HPV infection. From 20% to 60% of adult women in the United States are infected with one or another of the genotypes.

- More than 50% of sexually active people become infected with HPV at least once in their lifetime.

- HPV types 6 and 11 are associated most commonly (about 90%) with benign genital warts in males and females and with some cellular dysplasias of the cervical epithelium, but these lesions rarely become malignant.

- HPV types 6 and 11 have been associated with nasal, oral, conjunctival, and laryngeal warts. They can be perinatally transmitted and cause infantile laryngeal papillomas.

- HPV types 16, 18, 31, 45, and 56 may cause lesions of the vulva, cervix, and penis.

- Anal neoplasia occurs among homosexuals as a result of HPV infection in the anus.

Pathogenesis

- HPV has predilection for infection at the junction of squamous and columnar epithelium (eg: in the cervix and anus).

Additional Information:

- At the rectoanal junction, there's a transition from simple cuboidal epithelium to stratified squamous epithelium (See Junqueira, page 317).
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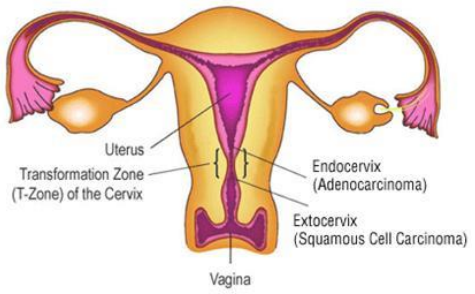

This figure shows the endocervix which is lined by mucous-secreting columnar epithelium and the exocervix which is lined by stratified squamous epithelium. HPV infection occurs at the junction between squamous and columnar epithelium, hence the name "Transformation Zone"

See Junqueira's Basic Histology, 13th edition, page471.

This sentence is taken from the slides "Pathogenicity and oncogenicity is not well understood".

- We've studied the oncogenicity of HPV with Dr. Mazin last semester. Since it's not mentioned in the slides, it won't be mentioned in the sheet. Refer to Sherris textbook, page

336.

- **Risk Factors for Acquiring a Genital HPV Infection**

•Young age (less than 25 years)

•Multiple sex partners

•Early age at first intercourse (16 years or younger) → the best time to give the vaccine is before the first sexual intercourse.

•Male or female partner has (or has had) multiple sex partners.

<u>Transmission</u>

Transmission through:
1- Direct skin-to-skin contact (Primary route), more specifically sexual contact with infected penis, scrotum, vagina, or anus.

- Anal involvement is seen mostly in homosexuals, especially ones with HIV.

- Contact with infected lesion can also lead to disease development.

- Perinatal (During the passage of the baby through the birth canal). The newborn usually develops oral or pharyngeal papilloma.

-Average incubation period is long (3 weeks to 1 year). So, a patient can be infected for a long time without the appearance of any symptom.

Common Symptoms of Genital Warts in Males & Females

•Genital warts are unsightly cauliflower-like growths (6, 11)

•Some types are considered “high risk” and can cause pre-cancerous lesions and can lead to cancer of the cervix, anus and other genital areas.(16,18)

•The symptoms may include single or multiple fleshy growths around the penis, scrotum, groin, vulva, vagina, anus, and/or urethra

•They may also include: itching, bleeding, or burning, and pain

•The symptoms may recur from time to time

Diagnosis

- HPV infection in the cervix causes enlargement of the nucleus and epithelial dysplasia. PAP smear followed by microscopic examination of the tissue can be used for diagnosis.
- Immunoassays for viral antigen detection.
- PCR for specific viral DNA detection.

HPV Treatment

- HPV treatment is currently either cytotoxic or surgical.

Cytotoxic treatment:
Topical → podophyllin, podophyllotoxin, 5-fluorouracil, and trichloroacetic acid.
Systemic interferon-alpha also may benefit.

Other treatments:
•Cryotherapy: freezing with super cold liquid or gas nitrogen

•Electrosurgery: using an electric current to remove warts

•Surgical excision

• Cervical and anal lesions may be treated with electrocautery but carcinoma may require radiation therapy or radical surgery.

Prevention

Gardasil

- Quadrivalent vaccine (prevents infection by HPV 6,11,16,18).
    - The first vaccine to prevent cervical cancer.

•Recombinant vaccine; inactive L1 proteins

•Approved for use in females aged 9-26
–Ideally, before becoming sexually active
•Three injections given over a six-month period
-Initial dose
-Second dose is given 2 months later
-Third and last dose is given 4 months after the second dose or six months after the initial dose
•It is administered in the upper arm or thigh (intramuscularly).

Cervarix
•designed to prevent infection from HPV types 16 and 18 (bivalent).