SHEET NO. 8

MICROBIOLOGY (Virology)

DOCTOR 2019 | MEDICINE | JU

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SCIENTIFIC CORRECTION :

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بسم الله الرحمن الرحيم

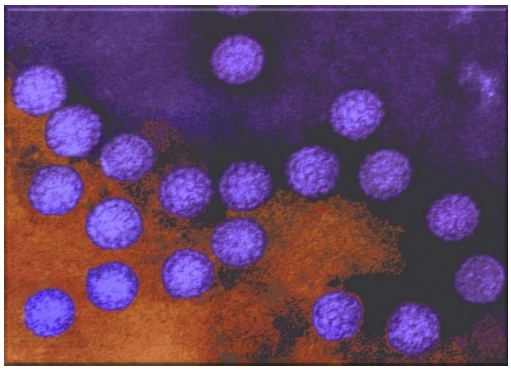
In this lecture we're going to discuss:

1. Polyomaviridae
2. Human papillomavirus
3. Pox viridae

Polyomaviruses

OVERVIEW

1. **Genome**: Double stranded circular DNA, 5-5.1kb
2. **Shape** : *Icosahedral* -> The capsid contains three structural proteins, *viral protein 1,2&3*. It contains 72 capsomeres (a capsid subunit). There are 5 VPs arranged around either VP2 or 3. In the cytoplasm, *VP2&3 transport VP1 near the nucleus* to assist in the virus assembly. Under the microscope, we can see VP1 particles aggregate simultaneously to form the capsid.(DNA virus -> the assembly occurs in the nucleus)



3. **Size :** around 40nm in diameter.
4. **Most know / important types:** JC virus, BK virus & the oncovirus Merkle cell polyomavirus (MCP causes cancer)

SUBTYPES

JC & BK viruses

(OVERVIEW): These two are among the most important subtypes of polyomaviruses. The infection is *acquired in early childhood and some people maintain a life-long antibody titer* (how much antibody an organism produced that recognise a certain epitope) for both JC & KB but not in high levels.
Both of these viruses *can be isolated from TOTALLY healthy cells* but they can be *associated with some diseases*, unlike the simian virus 40 (which is a subtype of polyomaviruses but *it's not known to be associated with any diseases*).

EPIDEMIOLOGY They are *very common* & according to some studies their seroprevalence can reach up to 70% globally.

TRANSMISSION & ENTRY: Primarily by respiratory inhalation or being exposed to body fluids like the blood, urine or oral secretions. The receptors for these viruses are α-2,6 sialic acid receptors & the entry is accomplished by clathrin-mediated endocytosis. (recall cytology? 🤢). There's a receptor called 5-HT2A serotonin receptor which is special for JC virus.

INFECTION: For immunocompetent individuals the infection is normally asymptomatic or can cause mild symptoms e.g., fever & nonspecific upper respiratory tract symptoms

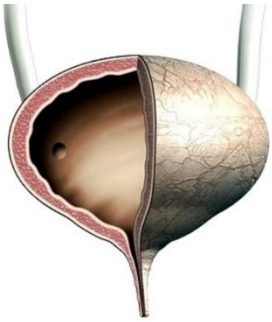
1. *JC virus* -> the primary site of infection is the *tonsillar stromal cells* because they're susceptible to the infection either in vitro or in vivo and because of their location in the upper respiratory tract (transmission)
2. *BK virus* -> it can replicate in the *tonsillar tissue* or the *nasopharyngeal tissues*, we can find an evidence of the presence of this virus so the initial route of infection may be inhalation

***B lymphocytes can a site of replication for both JC & BK viruses although they're less susceptible to the infection.**
***These viruses may remain latent for life with no symptoms in the cells of the kidneys, sites of the lymphoid tissue and there's evidence that JC may be latent in the cornea, unless there was some sort of immunity suppression.**

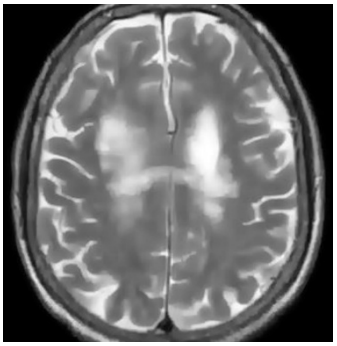
In cases of immune deficiency especially cellular immune deficiency, the latent viruses may be **activated** causing *viral URI* (upper respiratory tract infection), and in some cases this might cause manifestations like *cystitis (BK virus)* or *leukoencephalopathy (JC virus)*

Alright then, let's talk about those manifestations!

- BK virus reactivation can cause *cystitis* (haemorrhagic or nonhaemorrhagic), it may also cause *urethral stenosis* (narrowing of the urethra), in those cases people who receive bone marrow transplantation were at higher risk of developing the diseases. The virus was also associated with *nephropathy (polyomavirus associated nephropathy PVAN)* which has a 1-5% incidence on renal transplant recipients.



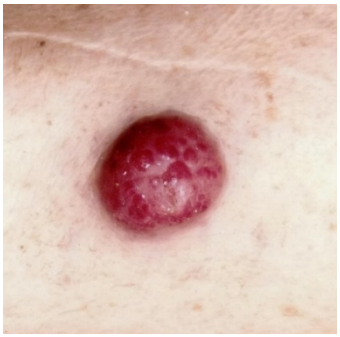
- JC virus reactivation is associated with *Progressive multifocal leukoencephalopathy*, it can be considered an opportunistic infection (occur more frequently & more severe in people with weakened immune system) and it includes a group of symptoms like motor dysfunction, visual problems & communicative impairment.
  The manifestations are hemiparesis (slight weakness & mild loss of strength), cortical blindness (loss of vision in normal-appearing eye because of a problem in the brain cortex, JC infection in this case) & progressive dementia.
  Before 1980s this infection was rare but after that and with the increase in AIDs cases it appeared more.
  Before, it was known to affect immunocompromised patients especially those with lymphoproliferative malignancies like lymphomas or leukaemia but then it started to appear in AIDs patients (5% of them).



JC virus is considered a **causative agent** of the disease, *it's constantly isolated from the brain tissues, as well as its DNA and antigens,* which we can detect using *immunohistochemistry techniques or in situ hybridisation* (recall molecular biology &histology) or we can use the histopathological examination -> *lytic infection of oligodendrocytes* which will cause focal or multifocal demyelination of the brain's white matter.

Merkle cell polyomavirus

This virus is an oncovirus meaning its infection causes cancer (**merkle cell carcinoma**) which is a very aggressive skin cancer that specially *affect those who have a fair skin, elderly people and immunocompromised individuals*. It occurs mainly on the areas of the skin that are *exposed to the sun* for example the head and neck.
The virus contribution to the disease was *discovered recently* & its prevalence is variable in different studies but there are other risk factors that contribute to the pathogenesis of merkle cell carcinoma.



TESTING

1. Histopathology changes that are associated with BK or JC viruses
2. Hybridisation for DNA molecules
3. Antigen detection (T antigen in merkle cell carcinoma)
4. MRI is the imaging modality of choice if PML was suspected. The confirmatory test is JCV DNA in the CSF or brain by PCR

TREATMENT

There's no licenced antiviral therapy for neither JCV nor BKV
For MCC: surgery, radiotherapy & chemotherapy.

Papillomavirus

OVERVIEW

Papillomavirus is everywhere, almost 100 of human papillomavirus have been identified (infect humans)

1. **Shape:** naked icosahedral
2. **Slides information:**

1. Family: papillomaviridae
2. Genus: alpha-papillomavirus
3. Species: Human papillomavirus -> members share form 60-70% identity
4. Type -> share 71-89% identity

5. Subtype -> share 90-98% identity
6. Variant

3. **Genome:** Double stranded circular DNA
4. **Size:** 40-55 nm in diameter
5. **Gene products:** 8 early genes (E1-E8) & 2 late genes (L1 & L2)

The gene products functions

1. **E1&E2:** have a relation to DNA replication
    - E2 has been demonstrated to repress the activity of the early viral promoters responsible for the expression of E6 & E7 oncogenic
2. **E4:** have roles in the alteration of cytoskeleton
3. **E5:** encodes membrane proteins with weak transforming activity
4. **E8:** its amino acid terminal consist of 22 amino acids that are representative of transferable DNA replication presser domain that control the viral copy number during the persistent infection
5. **E3:** gene function is not well understood yet
6. **L1&L2:** are structural proteins (viral capsid proteins)
    - L1 have sequence heterogeneity that is used for HPV typing (meaning that the differences (71-98) % between these viruses are mainly in this gene L1 gene)
    - L1 gene is the major target of molecular diagnostic assays of HPV

EPIDEMIOLOGY

1. HPV is related to cervical cancer; it contributes to almost 10% of the cases globally
2. Epidemiologic studies carried out in last 30-35 years refers that the persistent infection in oncogenic types of HPV followed by the HPV DNA integration of cellular genome is an *important precursor in the pathway that causes cervical neoplasia* (there are about 100 different types of HPV but only the oncogenic types are associated with cancers such as the cervical cancer, anal cancer and oropharyngeal cancer)
3. If we take all cervical cancer patients, the prevalence HPV related cervical cancer could exceed 90%, so it's serious risk factor of cervical cancer.
4. HPV is the most common cause of STIs especially in the united states. Risk factors for acquisition of HPV: early sexual debut, multiple sexual partners, Other STIs.

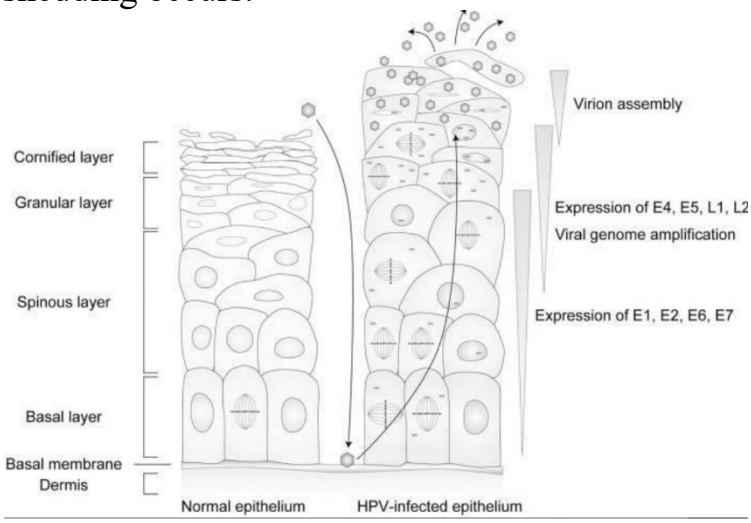
TRANSMISSION

1. Transmission occurs from males to females or vice versa by the virus shedding specially from males followed by the infection of cervical epithelial basal cells (**initiation of infection occurs in these cells**) … there is microabrasion mediate the reachment of HPV to the basal cells

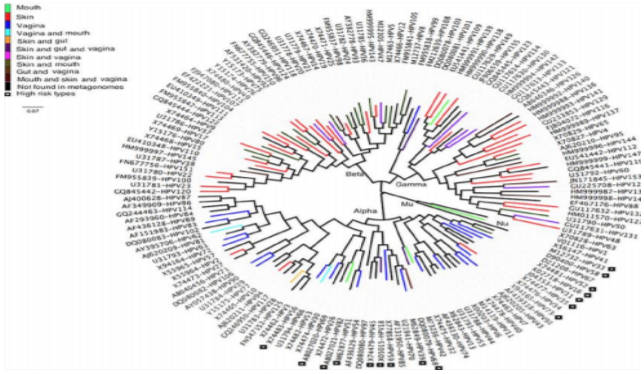
INFECTION

1. HPV is **unique by its infection to proliferative cells** (cells that have proliferative capability).
2. The HPV life cycle depends on the maturation of the epithelial basal cells.
3. After the infection of HPV of the basal epithelial cell layer, HPV will establish an episome of about 50 copy for each cell and these episomes replicate with the cellular the DNA, so the HPV episome will replicate once the DNA replication during cell division starts, then the HPV episome will migrate from the basal layer into the supra basal layer and the differentiation of cells occurs and new group of protein will be expressed, when it reaches the supra basal layer the HPV infected cell will enter the S phase and will produce hundreds to thousands of HPV DNA for each cell. After viral DNA synthesis the viral proteins including the capsid proteins will assemble the complete viral particle, the virions will be produced and they will be released into female genital tract when the epithelium shedding occurs.

This figure illustrates the initiation of infection in basal cells and different phases of differentiation of basal cells either mucus membranes or the epithelium accompanied with expression with a certain set of genes at each phase until it's the shedding occur to initiate another cycle



Papillomaviridae-Classification



Important note: there are some types associated with infections in certain places within the body

Clinical manifestation of HPV infections

1. HPV virus is the major cause of warts in cutaneous infection (B9 HPV), larinks, anogenital tissue
2. Cutaneous infections occur in all people whether they were immunocompetent or immunocompromised patients and it could cause malignant lesions.
3. As a rule, plantar warts are exclusively caused by **B9 HPV**

4. There is an association between the HPV types and the type of warts for example:
   - Common warts are caused by HPV types 2,4,26,27,29 and 57
   - Flat warts are caused by HPV types 3,10,28 and 29
   - Plantar warts are caused by HPV types 1,2 and 4
   - There is a disease associated with inherited disorder in cell mediated immunity epidermodysplasia verruciformis associated with HPV types 2,3,5,8,9,10,11,14,15,17,19,20,25,36,37,46,47 and 50
   - In oral mucosa it is caused by B9,13,32 types
   - There are low risk types of HPV associated with laryngeal papillomatosis which are HPV 6 and 11
   - Also, there are some types associated with head and nick cancer including HPV 16,18,30
   - In genital mucosa warts such as Condyloma acuminata are caused by 6,11,42,43,44,54
   - Low risk HPV types: 6,11,40,42,43,44,54,61,70,72,81
   - Intermediate risk HPV types: 26,53,66
   - High risk HPV types (genital high-risk types): 16,18,31,33,35,39,45,51,52,56,58,59,68,73 and 82

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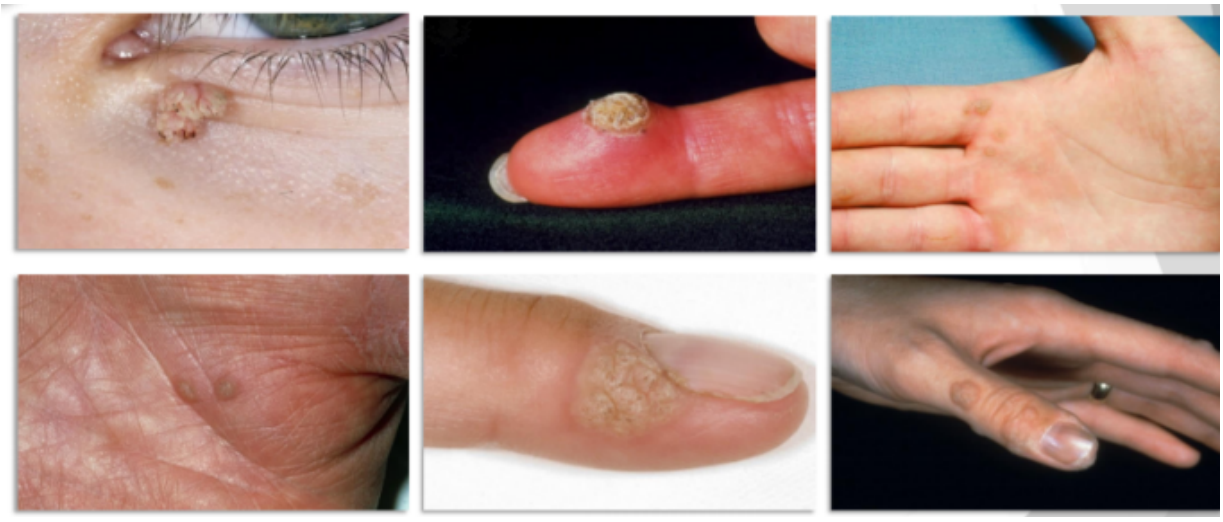


5. In cervical cancer (cervical squamous cell carcinoma) more than **99% of the cases is caused by infection of squamous cells** by oncogenic type of HPV

6. HPV viruses may contribute in adenocarcinoma of uterine cervices
7. In high-risk HPV lesions, the **transcriptional activity is present**, E6&E7 oncoproteins and certain host factors involved in the transformation of cells and the development of cervical cancer
8. E6 cause **B35 & BAK degradation and activation of SRC & telomerase**
9. E7 **degrade the retinoblastoma proteins releasing E2F transcription factor and cause upregulation** of cellular protein B6NK4A.

There are several evidences support the rule of E6&E7 in the pathogenesis of cervical cancer from studies that carry out demonstration of suppression of E6&E7 promoter (inhibition of E6&E7 expression) which result in the elimination of proliferation of cancer cells in vitro

How to differentiate between high risk and low risk types and what cause increase the expression of E6&E7?

- E2 is a negative regulator of the expression of E6&E6, during the integration of HPV episome in the host cell DNA, distraction of E2 and then that negative regulation on E6&E7 will be repressed that associate with the increase in oncogenic potential
- The levels of expression of E6&E7 genes determine whether the HPV is high or low risk
- Other gene regions could have transforming capabilities such as E5, but still E6&E7 control the oncogenic potential of HPV types

10. Smoking used to be the most common factor for oropharyngeal cancer, but recently (20-30 years) HPV as a risk factor for oropharyngeal cancer is on the increase because of the increased oral sexual practices which increases the risk of acquiring high risk types in the oral cavity. (it might have become the most common risk factor in some countries)

11. It might be associated with penile cancer

DIAGNOSIS

1. PAP smears to detect premalignant lesions in the cervix to diagnose low grade dysplasia and carcinoma in-situ (PAP smears revolutionized screening of cervical carcinomas which is the second most common cancer in women after breast cancer)
2. Depends on appearance of lesions
3. Typing
4. PCR
5. Histopathologic examination

6. Immunohistochemistry stains for certain high-risk types such as p16 for HPV 16

TREATMENT

There is no treatment if the infection creates episomes (plasmid combined with cellular DNA) but it can be managed. For example: oral squamous papilloma, warts, or lesions can be managed by

- surgical removal
- liquid nitrogen
- laser vaporization
- chemical agents (such as podophyllin toxin).

But there is NO cure

PREVENTION

two vaccines were approved in 2006 and 2007, one protects against 4 types (6,11,16&18), and the other protects against (16 and 18)

- Long term effects of these vaccines are undetermined, and they mainly protect against genital infections to prevent cervical cancer (mainly made of complement structural protein which is L1 protein in HPV)
- Vaccines are given at early ages (such as 12,13 in some countries) to young females

- Some notes:
  - Transmission of HPV still needs more investigation
  - The disease is worldwide and (minute 33:11?????????)

Poxviridae

OVERVIEW

1. Most famous member of the poxviridae family is the smallpox virus (also called variola) which used to cause fatal diseases, but *it was the first and only infectious disease in humans to be eradicated*. There were multiple attempts to eradicate polio which has decreased in number of infections significantly but it hasn't been eradicated yet.

2. Last natural case of smallpox was in southern Somalia in 1977 of a minor. Smallpox was one of the first infectious diseases to get a vaccine (remember Edward Jenner's story in immunology)
3. The variola virus is part of the Poxviridae family (another member of the Poxviridae family causes mulloscum contagiosum which is characterized by skin lesions but isn't as bad as smallpox)

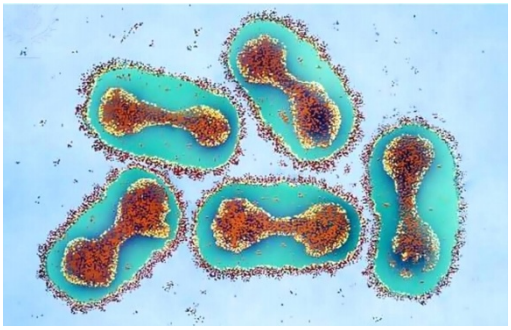
- There are two types of variola viruses which are differentiated by RFLP (they are very close to each other and we could say that they are variants of each other)
    a. *Variola major*: mortality rate is 30%
    b. *Variola minor*: which causes a milder form of the disease with the same manifestations but mortality rate doesn't exceed 1% which could be caused by shock or cardiovascular collapse.

4. Smallpox is a **potential bioterrorism agent** because there is no vaccination or natural immunity in smallpox (vaccination stopped after mid 70's) so young people don't have immunity to the virus.
5. The virus is only found in two places in the world: CDC labs in Atlanta Georgia, and in a lab in Siberia. The virus might be found in other labs in other countries)

What are the factors that led to successful eradication of smallpox? (Important)

1. Single antigenic type
2. No asymptomatic cases
3. No animal reservoir
4. The presence of an effective vaccine
5. Other factors such as: disease severity and psychological effects (which encouraged WHO campaigns to eradicate smallpox and people cooperated to help eradicate the disease as the disease was fatal and/or causes many disfigurements)

6. **Shape:** Enveloped, brick shaped with no obvious symmetry (complex)
7. **Genome:** DNA virus, very large
8. **The only DNA virus to replicate in the cytoplasm** (it has enough enzymes packaged to initiate RNA synthesis in cytoplasm)



TRANSMISSION

Smallpox can be transmitted by infection of mucosal cells in upper respiratory tract then goes to regional lymph nodes and causes transient viremia and spreads throughout the body and multiplies in various types of cells then returns to the blood to cause intense viremia and

goes to the skin epithelial cells to cause lesions that spread centripetally (periphery -> central axis of the body) (moving or tending to move towards a centre.)

CLINICAL FEATURES

1.The rash starts from macules -> papules -> vesicles -> pustules -> permanent bookmarks that heal with permanent scars (lifelong disfigurements), (in contrast to chickenpox where the rash is centrifugal -> basically from trunk -> periphery)

2. Mollascum contagiosum
   - Most important feature is that it is self-limited (spontaneous resolution after a couple of months)
   - Papules that are umbilicated (has a dent in the center like the bellybutton) that can appear anywhere on the body.
   - Can be treated by cidofovir, but it is self-limiting so it doesn't require treatment