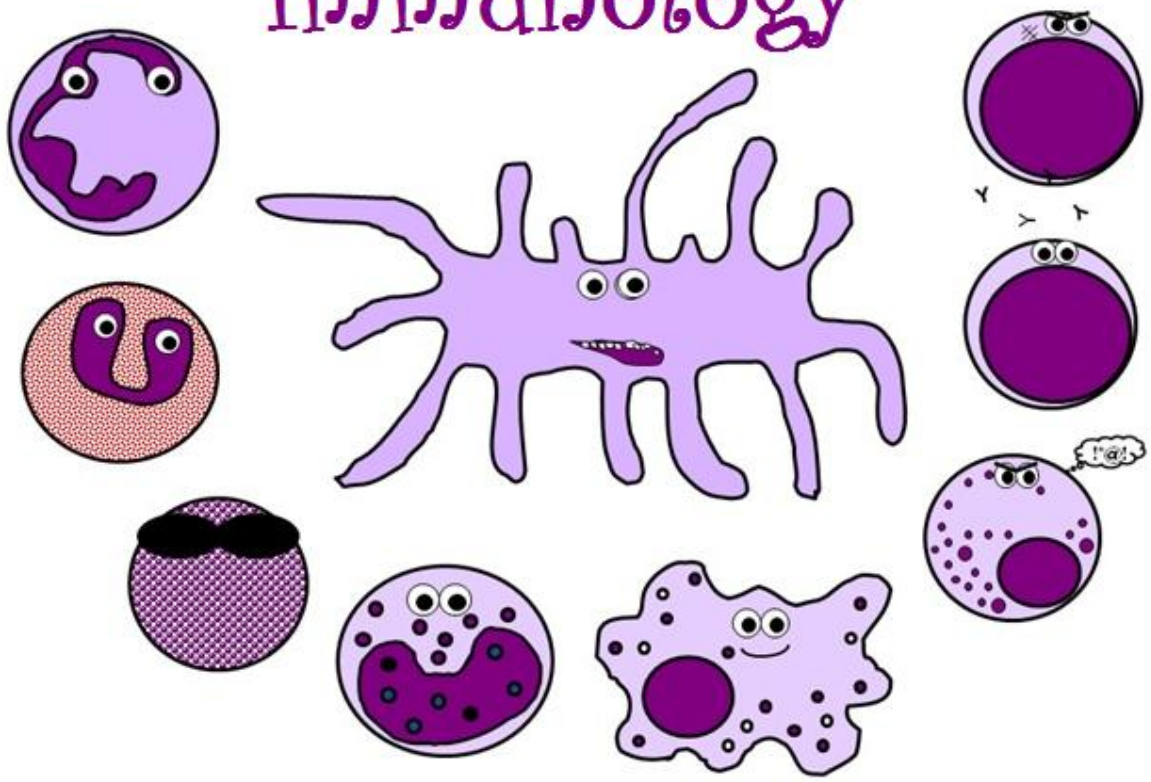
Immunology



✔ Sheet ○ Slides

Number: 11

Subject: congenital asplenia

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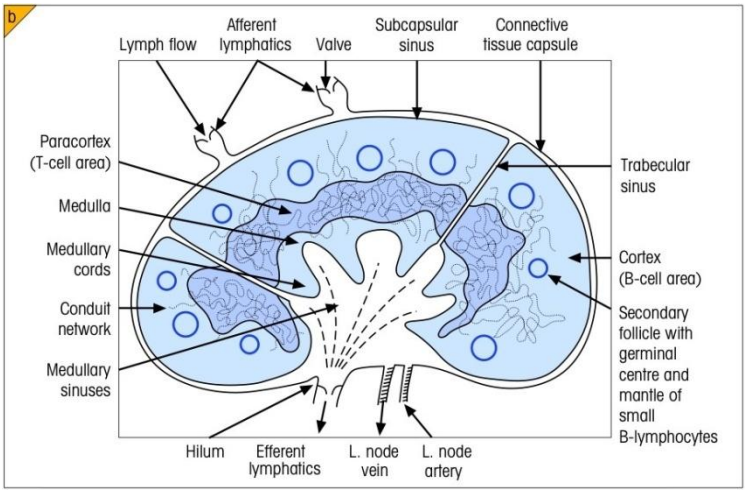
00/00/2016

-Hey everyone, this is my first sheet I hope you will like it :)

-So today's lecture is about congenital asplenia, we will go through a brief introduction, then we will attach the case study included pages from the book, after that we will g through the doctor notes inshallah.

Introduction:

So, what do we know the spleen from an anatomical and histological view so far?



-The spleen is a secondary lymph organ.

-The splenic artery enters the spleen through the hilum,then it will give branches inside the spleen, known as the trabecualr arteries,then they will become central arteries.during the course of the central artery, it will be surrounded by a sheath, also known as the periarteriolar sheath(this contains the T cells and their APC's, the interdigitating dendritic cells.After that, we notice that this sheath gives some follicles (by expansion around the artery), these follicles contain mainly the B cell population and their the follicular dendritic cells (their APC's)..This is all called the White puplp of the spleen.Now we also have a red pulp, but before that there is waht's called the marginal zone, contains a lot of macrophages (for blood filtration).The red pulp consists of two parts; the splenic cords (it's an open circulation here) after this the blood will go to the other part which is the

(sinusoids)..here the defective and old RBC's as well as platelets will be filtered as well.

So the spleen filters the blood from defective RBC's and platelets, microbe filtration and it can carry on an immune reaction although it was thought to be of no significant function !

-The doctor didn't mention this, it's only for you to recap some info about the spleen.

** Congenital means inborn and inherited, while asplenia is the lack of spleen(physical absence or functionally defective),

and in this case we will have a look at a family whose more than half of their children were born asplenic(without spleen) :(

The introduction is over

Let's start by the required pages from the book:

CASE 30 Congenital Asplenia

The role of the spleen in immunity.

The adaptive immune response occurs mainly in the peripheral lymphoid tissues—the lymph nodes, the gut-associated lymphoid tissue, and the spleen (Fig. 30.1). Pathogens and their secreted antigens are trapped in these tissues and presented to the naive lymphocytes that constantly pass through. Microorganisms that enter the body through the skin or the lungs drain to regional lymph nodes, where they stimulate an immune response. Microorganisms and food antigens that enter the gastrointestinal tract are collected in the gut-associated lymphoid tissue. Microbes that enter the bloodstream stimulate an immune response in the spleen.

The spleen is organized to accomplish two functions (Fig. 30.2). In addition to being a peripheral lymphoid organ, it acts as a filter of the blood to remove aged or abnormal red cells and other extraneous particles that may enter the bloodstream, including microorganisms. In the absence of a functioning spleen, these aged and abnormal red blood cells can be seen in a peripheral blood smear in the form of pitted red blood cells and Howell–Jolly bodies (nuclear remnants in red blood cells that are usually removed by the spleen) (Fig. 30.3).

The lymphoid function of the spleen is performed in the white pulp, and the filtration function by the red pulp. Many microorganisms are recognized directly and engulfed by the phagocytes of the red pulp. Others are not removed efficiently until they are coated by antibodies generated in the white pulp. In experimental animals, an immune response (as measured by

This case was prepared by Raif Geha, MD, in collaboration with Itai Pessach, MD.

Topics bearing on this case:

- Circulation of lymphocytes through peripheral lymphoid tissues
- Toxoid vaccines
- Hemagglutination tests

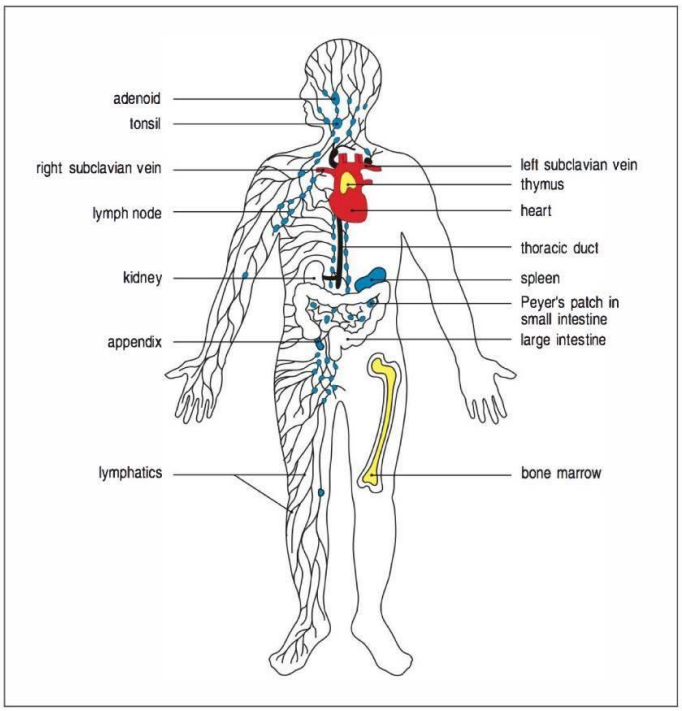


Fig. 30.1 The distribution of lymphoid tissues in the body. Lymphocytes arise from stem cells in bone marrow, and differentiate in the central lymphoid organs (yellow)—B cells in bone marrow and T cells in the thymus. They migrate from these tissues through the bloodstream to the peripheral lymphoid tissues (blue)—the lymph nodes, spleen, and mucosa-associated lymphoid tissues such as tonsils, Peyer's patches, and appendix. These are the sites of lymphocyte activation by antigen. Lymphatics drain extracellular fluid as lymph through the lymph nodes and into the thoracic duct, which returns the lymph to the bloodstream by emptying into the left subclavian vein. Lymphocytes that circulate in the bloodstream enter the peripheral lymphoid organs, and are eventually carried by lymph to the thoracic duct, where they reenter the bloodstream.

antibody formation) can be detected in the white pulp of the spleen about 4 days after the intravenous injection of a dose of microorganisms. The clearance of antibody- and complement-coated bacteria or viruses by the phagocytic cells of the red pulp of the spleen is very rapid. Rapid clearance from the blood is important because it prevents these bacteria from disseminating and causing infections of the meninges (meningitis), the kidney (pyelonephritis), the lung (pneumonia), or other distant anatomical sites.

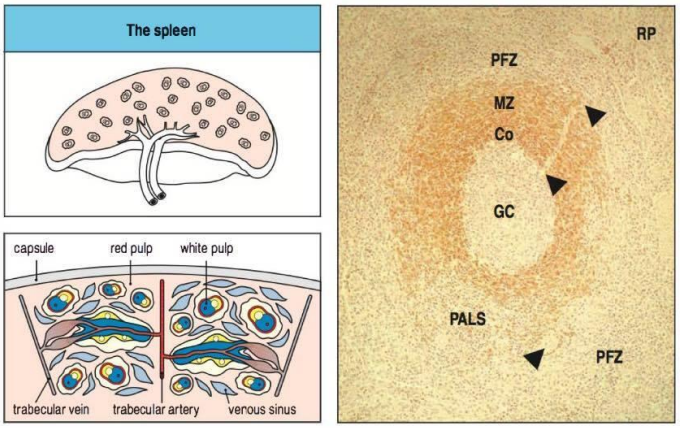


Fig. 30.2 Schematic views and light micrograph of a section of human spleen. The schematic at top left shows that the spleen consists of red pulp (pink areas), which is a site of red blood cell destruction, interspersed with the lymphoid white pulp. An enlargement of an area of white pulp is shown below. The follicle (yellow in the middle panel) and T-cell areas (blue) are surrounded by the perifollicular zone (PFZ) (palest yellow). The light micrograph on the right shows a transverse section of white pulp immunostained for mature B cells. The follicular arteriole emerges in the periarteriolar lymphoid sheath (PALS) of T cells (lower arrowhead in the bottom panel), traverses the follicle, goes through the marginal zone and opens into the perifollicular zone (upper arrowheads). Co, follicular B-cell corona; GC, germinal center (activated B cells); MZ, marginal zone (B cells); RP, red pulp; arrowheads, central arteriole. Photograph courtesy of N.M. Milicevic.

Fig. 30.3 The arrow marks a red blood cell with Howell–Jolly body. These inclusions are formed by the retention of nuclear remnants in red cells, which are usually removed by the spleen but can be found in patients with asplenia or significantly decreased splenic function.

Bacteria enter the bloodstream all the time, such as when we brush our teeth or when we have a local infection, for example of the skin or middle ear. Normally, these bacteria are disposed of efficiently by the spleen. When, for one reason or another, the spleen is not present, serious, even fatal, infections occur.

The case of Susan Vanderveer: a fatality because of an absent spleen.

Mr and Mrs Vanderveer owned a farm in the Hudson Valley in lower New York State. They were both descended from Dutch settlers who came to the Hudson Valley in the mid-17th century. There were multiple consanguineous marriages among their ancestors, and Mr and Mrs Vanderveer were distantly related to each other. At the time of this case, they had five children—three girls and two boys. Their youngest daughter, Susan, was 10 months old when she developed a cold, which lasted for 2 weeks. On the 14th day of her upper respiratory infection, she became sleepy and felt very warm. Her mother found that her temperature was 41.7°C. When Susan developed convulsive movements of her extremities, she was rushed to the emergency room but she died on the way to the hospital. Post-mortem cultures of blood were obtained, and also from her throat and cerebrospinal fluid. All the cultures grew *Haemophilus influenzae*, type b. At autopsy Susan was found to have no spleen.

Susan Vanderveer, age 10 months, dead on arrival in Emergency.

At the time of Susan's death her 3-year-old sister, Betsy, also had a fever, of 38.9°C. She complained of an earache, and her eardrums were found to be red. She had no other complaints and no other abnormalities were detected on physical examination. Her white blood count was 28,500 cells μl^{-1} (very elevated). Cultures from her nose, throat, and blood grew out *H. influenzae*, type b. She was given ampicillin intravenously for 10 days in the hospital and was then sent home in good health. Her cultures were negative at the time of discharge from the hospital. She was seen by a pediatrician on three occasions during the following year for otitis media (inflammation of the middle ear), pneumonia, and mastoiditis (inflammation of the mastoid bone behind the ear).

Betsy Vanderveer, age 3 years, presents with severe H. influenzae infection.

David, Susan's 5-year-old brother, had been admitted to the hospital at 21 months of age with meningitis caused by *Streptococcus pneumoniae*. He had responded well to antibiotic therapy and had been discharged. Another occurrence of pneumococcal meningitis at 27 months of age had also been followed by an uneventful recovery after antibiotics. He had had pneumonia at age 3½ years. At the time of Susan's death he was well.

The two other children of the Vanderveers, a girl aged 8 years and a newborn male, were in good health.

All the Vanderveer children had received routine immunization at ages 3, 4, and 5 months with tetanus and diphtheria toxoids and killed *Bordetella pertussis* to protect against tetanus, diphtheria, and whooping cough, which are three potentially fatal diseases caused by bacterial toxins (Fig. 30.4). Serum agglutination tests were used to test their antibody responses to these and other immunogens. Samples of serum from both Betsy and David caused hemagglutination (the clumping of red blood cells) when added to red blood cells (type O) coated with tetanus toxoid. Hemagglutinating antibodies against tetanus toxoid were seen at serum dilutions of 1:32 for both Betsy

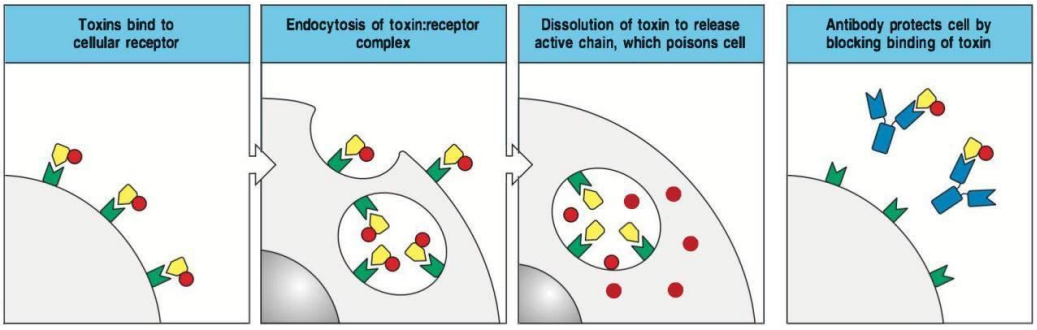
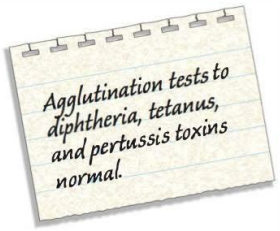


Fig. 30.4 Neutralization by antibodies protects cells from toxin action. Secreted bacterial toxins usually contain several distinct moieties. One piece of the toxin must bind a cellular receptor, which allows the molecule to be internalized. A second part of the toxin molecule then enters the cytoplasm and poisons the cell. In some cases, a single molecule of toxin can kill a cell. Antibodies that inhibit toxin binding can prevent, or neutralize, these effects. Protective antibodies can be generated by subcutaneous immunization with toxoids. Toxoids are toxins rendered harmless by treatment with denaturing agents, such as formalin, which destroy their toxicity but not their ability to generate neutralizing antibodies. In the case of the DPT vaccine, the killed *Bordetella pertussis* cells act as an adjuvant, which enhances the immune response to all components of the vaccine by delivering activating signals to antigen-presenting cells.

and David, and were found at a similar titer in their 8-year-old sister. All three children were given typhoid vaccine subcutaneously, and 4 weeks later samples of their sera were tested for the ability to agglutinate killed *Salmonella typhosa*. The results indicated a normal immune response. David had an agglutination titer of 1:16, Betsy 1:32, and their normal 8-year-old sister 1:32. All three children were given 1 ml of a 25% suspension of sheep red cells intravenously. David had a titer of 1:4 for hemagglutinating antibodies against sheep red blood cells before the injection. He was tested again 2 and 4 weeks later and there was no increase in titer. Betsy had an initial titer of 1:32 and her titer did not increase either. The 8-year-old normal sister had a preimmunization titer of 1:32. She was tested 2 and 4 weeks after the immunization, when she was found to have a hemagglutinating titer of 1:256 against sheep red blood cells.



All the children and their parents were injected intravenously with radioactive colloidal gold (^{198}Au), which is taken up by the reticuloendothelial cells of the liver and spleen within 15 minutes after the injection. A scintillation counter then scans the abdomen for radioactive gold. The pattern of scintillation revealed that Betsy and David had no spleens (Fig. 30.5).

Asplenia and splenectomy.

Significant impairment of splenic function can be either congenital, where the spleen is absent or dysfunctional at birth, or acquired as a result of conditions that damage the spleen such as trauma or sickle-cell anemia, and which often lead to its surgical removal (splenectomy). Congenital asplenia is further divided into two main categories. The more prevalent is syndromic asplenia, in which the lack of splenic tissue is part of a more complex genetic syndrome affecting other systems as well. In these syndromes splenic defects are usually associated with significant heart defects and heterotaxia, a condition in which malformations arise as a result of lateralization defects of organs in the thorax and the abdomen. Several human genes, including *ZIC3*, *LEFTYA*, *CRYPTIC*,

ACVR2B, and *CFC1*, all of which have important roles in directing lateralization, have been shown to be associated with these syndromes.

The second category is isolated congenital asplenia. This is a group of conditions in which the only abnormality is the lack of splenic tissue. Only a relatively small number of cases of true isolated congenital asplenia have so far been described. Most of these cases were diagnosed after episodes of overwhelming pneumococcal infections, either *post mortem* or while screening family members of affected individuals, as in the Vanderveer family. The genetic defect causing asplenia has not yet been identified. Most familial cases described so far follow an autosomal dominant pattern of inheritance, although families with an autosomal recessive or X-linked pattern of inheritance have also been reported.

The Vanderveer family is unusual in that three of their first four children were born without a spleen. After the events described in this case, the Vanderveers had three more children. One of the boys and the girl were also born without a spleen; the other boy had a normal spleen. This family provides us with an uncomplicated circumstance in which to examine the role of the spleen. The major consequence of its absence is a susceptibility to bacteremia, usually caused by the encapsulated bacteria *Streptococcus pneumoniae* or *Haemophilus influenzae*. This susceptibility is caused by a failure of the immune response to these common extracellular bacteria when they enter the bloodstream.

Surgical removal of the spleen is quite common. The capsule of the spleen may rupture from trauma, for example in an automobile accident. In such cases, the spleen has to be removed very quickly because of blood loss into the abdominal cavity. The spleen may also be removed surgically for therapeutic reasons in certain autoimmune diseases, or because of a malignancy in the spleen. After splenectomy, patients, particularly children, are susceptible to bloodstream infections by microorganisms against which they have no antibodies. Microorganisms against which the host has antibodies are removed quickly from the bloodstream by the liver, where the Kupffer cells complement the role of the red pulp of the spleen. Antibodies against the encapsulated bacteria that commonly cause bloodstream infections persist for a very long time in the bloodstream of exposed individuals, even in the absence of a spleen (for reasons that are not fully understood). Adults who already have antibodies against these microorganisms are therefore much less vulnerable to bacteremia than children who have not yet developed such antibodies. Fortunately, effective vaccines against both *S. pneumoniae* and *H. influenzae*, type b, have been developed, and are now part of the routine vaccinations given to many children worldwide, thus protecting asplenic children from some of the severe infections to which they are prone. Nevertheless, specific precautions, including prophylactic antibiotic treatment, are recommended to most individuals with an absent or non-functional spleen.

Fig. 30.5 A scintillation scan of the abdomen after intravenous injection with radioactive colloidal gold (^{198}Au) reveals that Betsy and David Vanderveer have no spleens. The top panel shows an abdominal scan of Betsy's mother. The large mass on the left is the liver and the small mass on the right is the spleen. The reticuloendothelial cells of both liver and spleen take up the labeled gold within 15 minutes after the injection. No spleen is seen in either Betsy (middle panel) or David (bottom panel).

Questions.

1 *Nicholas Biddleboy, a 5-year-old boy, has had his spleen removed after a sledding accident, during which both he and his sled struck a tree trunk. In the emergency room of a nearby hospital, it was determined that his spleen had ruptured. The surgeon, after removal of a spleen that had indeed ruptured, calls you for an immunology consultation. What do you advise?*

2 *Why did David and Betsy have normal responses to the typhoid vaccine but not to the sheep red blood cells?*

3 *The Vanderveer family is unique in the medical literature. The parents, who were distantly related, were normal and had normal spleens. Five of their eight children were born without spleens. Of these, only Betsy subsequently had children—four boys and one girl. They are all normal and have spleens. What is the inheritance pattern of congenital asplenia in this family? According to Mendelian laws how many of the eight Vanderveer children would be expected to have no spleen?*

And now let's move to the doctor notes..

-Congenital asplenia; to be born lacking a spleen.

-It follows an AR mode of inheritance(there is also autosomal dominant (AD) and X-linked modes of inheritance regarding congenital asplenia), but the information we have about the exact genes involved in the AR mode is still scarce and not exact.

-The lymphatics are of two groups;

*Primary(central): such as the bone marrow and the thymus.

*Secondary: lymph nodes,the spleen, mucosa associated lymphatic tissues (MALT),tonsils,..

-The red pulp is for RBC's, platelet filtration.. while the white pulp is for microbes filtration and establishing an immune attack.

-The parents are relatives (consanguineous marriage) ..What's the importance of this?

-Consanguineous marriages contribute to accumulation or pooling of the recessive genes, so that in the families that have many 'inbreeds' they may show some rare diseases.

**The doctor said that the Ashkenazi Jews for example are known for this type of marriages, so that the scientist tend to study them a lot; because they have many rare diseases that aren't found commonly in other populations.

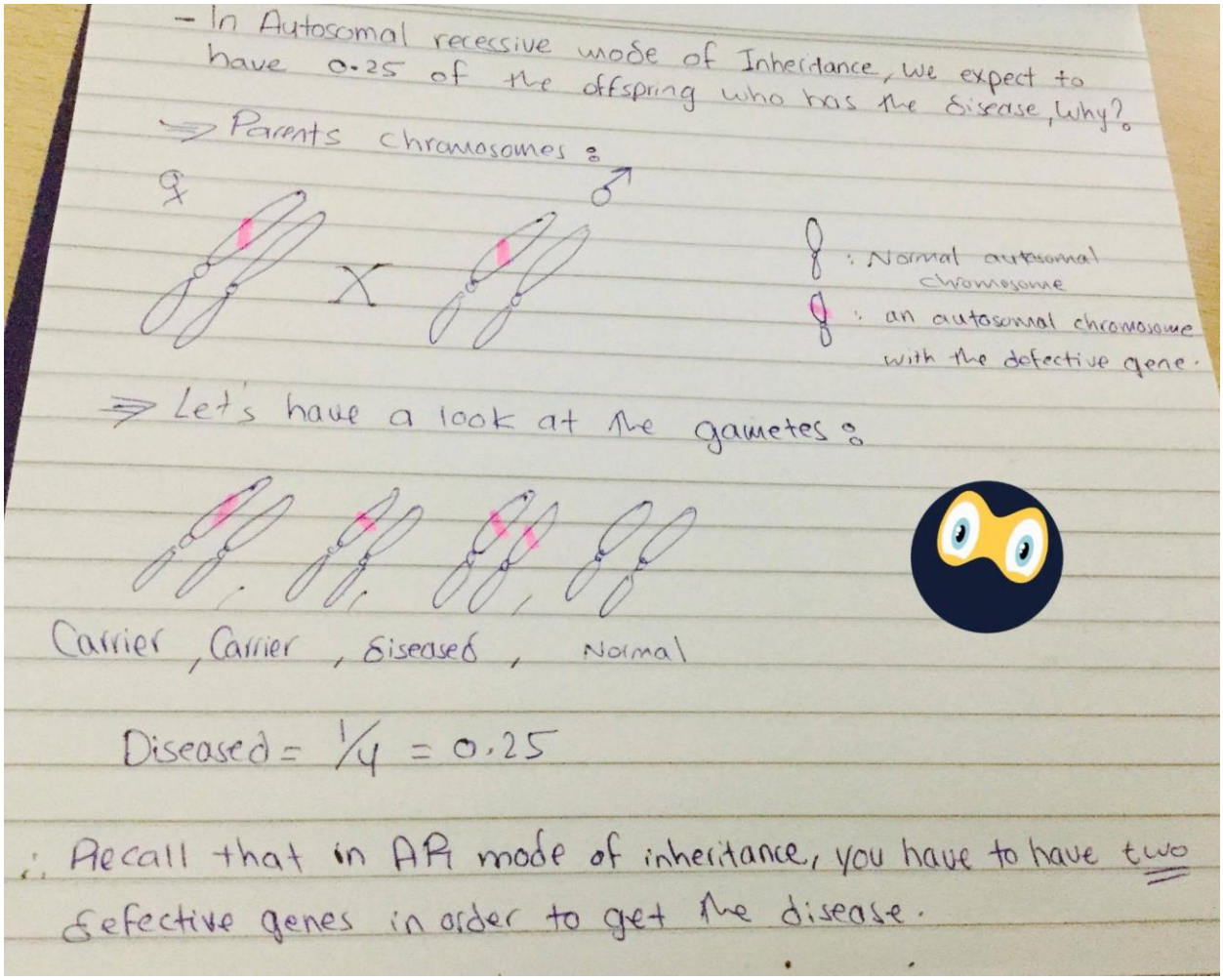
**Why is it of AR mode of inheritance not AD?

-it can't be autosomal dominant, because the parents had spleens,(they are carriers).

**Why not X-linked?

-Because the boys and girls are affected, if it was X-linked autosomal recessive, then the boys will show the disease more than the girls(if shown at all).

** You can have a look at this illustration showing how we get the result.



-As we can see, the percentage of the offspring that is expected to show the disease is only 0.25 but they had 5/8 (0.625) WHY?

because the number of children (n value) is small.. if they had 100 children :P then the number will be around 25..

-Why did we use the sheep RBC's?

1-becasue it's available and easy to obtain.. 2-we had to give them foreign RBC's intravenously(IV) to see the spleen response.

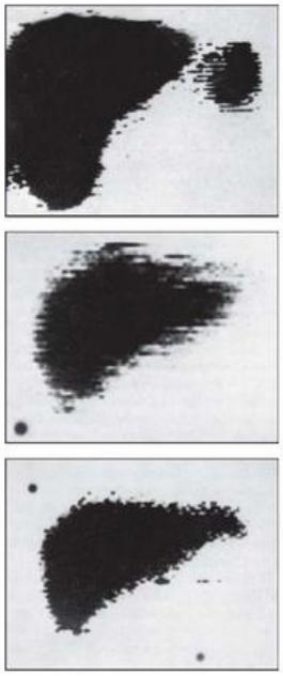
-What's the difference between the IV and SC vaccination in such case?

-SC vaccination is meant mainly to check the lymph nodes response, while if we give IV vaccination, then it's meant to check the spleen response(An increased number of antibodies indicates a positive response).

-What's special about S.pneumonia and H.influenza type B?

*Both are encapsulated bacteria and require antibodies to be attacked properly by the immune system.WHy? -the doctor said previously that they are a little but slippery and that the antibodies are thus required.

-The doctor mentioned something that isn't mentioned in the book, it's about the scintillation scan using radioactive gold.He listed some newer and practical techniques (will be mentioned in the next page) because the gold scan is a little bit invasive and also the gold is expensive :P



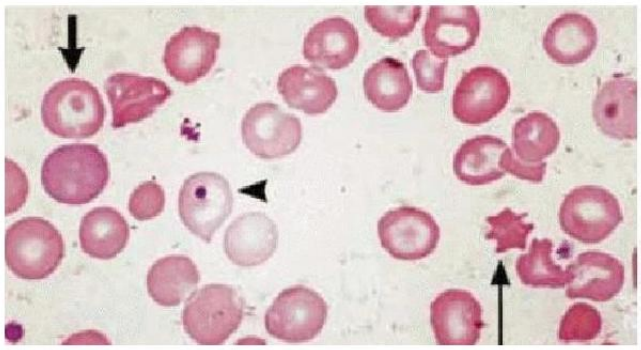
A scintillation scan of the abdomen after IV injection
With radioactive colloidal gold Au^{198}

Physical examination

CT, MRI, ultrasonography

Technetium ^{99}Tc Imaging

Peripheral blood film



-Regarding the blood film;

*We know that the spleen gets rid of the defective RBC's, so without a spleen they will appear in the blood film.We can see target cells(up and to the left) , acanthocytes(down and to the right) and Howell-Jolly bodies(in the middle)- they are remnants of the nuclear DNA.

-The doctor also discussed the internalization of the microbes or even their toxins; first of all there has to be a receptor- and here we can notice the importance of the antibodies: they bind the microbe/toxin and eases the process of its phagocytosis by the reticuloendothelial cells ***(ospsonization)*** and also they (neutralize) the microbes/toxins by masking the part of the microbe that will bind to the receptor, and thus they will prevent it from entering the cell as well***(Neutralization).***

-What's the toxoid?

-A bacterial toxin (usually an exotoxin) whose toxicity has been attenuated or inactivated, by heat treatment or by chemicals(such as formalin),while the immunogenicity(the ability of this toxin to induce an immune response against it) is maintained.The body will carry on a normal immune response as if it wasn't attenuated.

-What's MMR vaccine?

*Measles, mumps and rubella vaccine.

-What are the reticuloendothelial (RE)cells?

*They're group of cells having the ability to take up and sequester inert particles and vital dyes; such as macrophages.

-The doctor explained the mechanism of titration as well, let's have a quick look(just know the general idea).

-Let's say that the patient 's serum contains antibodies against a specific antigen of the RBC's...now we take the patient serum only(no RBC's) and we prepare some test tubes with increasing number of the antigens . Now when we add the serum-which contains the antibodies- it will cross link the antigens of the 'prepared' RBC's and an agglutination reaction will occur (we can easily tell if the reaction happens).The first tube will contain the same amount of the serum so that the ratio is (1:1).. the second tube will have the same amount of the serum but the antigen(RBC's with the antigen) is doubled so it will be (1:2)..then (1:4)..(1:8)..(1:16)..(1:32)...(1:64) and so on..

now as we dilute the serum, while the antibodies concentration is constant.. there will be less agglutination in each increasing tube(i.e. 1/8 will have more

agglutination than 1/16 and 1/32)WHY? -Because in 1/8 we have less antigen (less dilutes).

(As we dilute more, the agglutination reaction start to diminish)

Finally, if at 1/128 ,for example, we notice that the solution is clear, we look at the one before this tube directly which has agglutination ....>it will be 1/64.. BINGO, You have just found your titer.(so we take the ratio of the tube that is just before the tube that has no agglutination at all).Hope it's clear now :)

-Now we look at the pathology of the disease to help us interpret our results(for example high titers may indicate recent infection , and low titers may indicate old infection).(Figure : 1)

-Why is the spleen more important in children compared to adults?

*Because at the begging of your life, you will be exposed to a larger amount of antigens , so that it's important to have an intact immune system,while if you lose your spleen after 30 years for example, then your body have already faced many antigens and had memory cells and immunoglobulins against them.

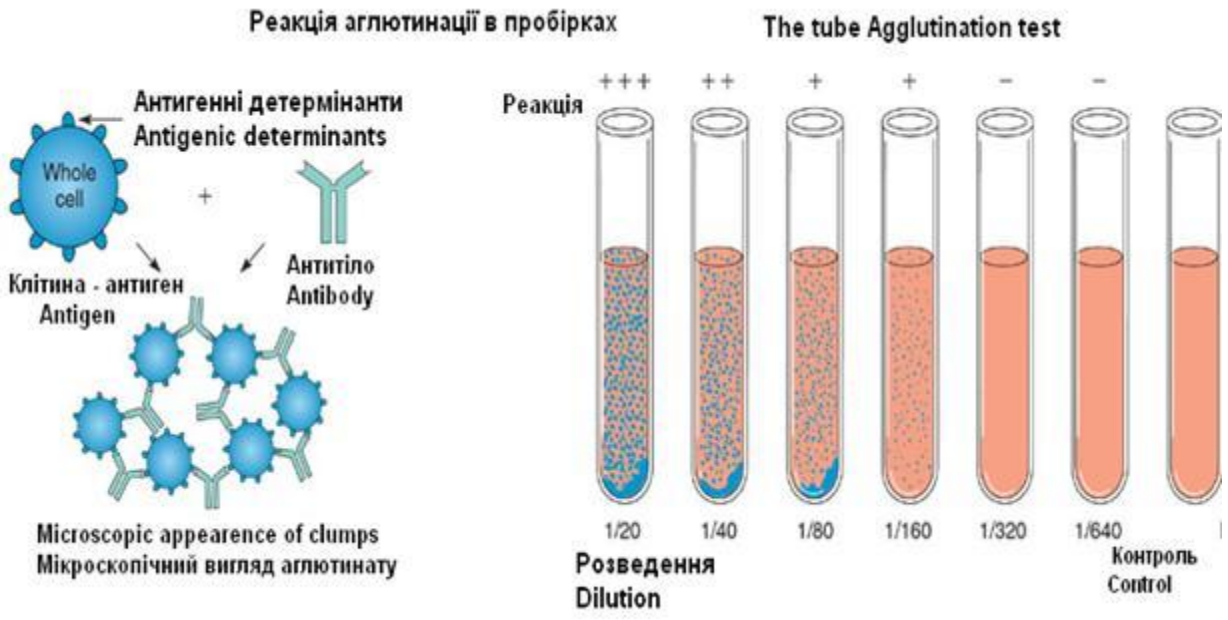


Figure 1: Our titer here is 1/160.

Questions:

Why did brother and sister respond to typhoid vaccine but not sheep RBC?

Typhoid vaccine is given s.c. But sheep RBC given IV.

Mode of inheritance of congenital asplenia?

Autosomal recessive

How many kids out of 8 were expected to have the condition?

2

Betsy the sister married a normal man, what is the condition of the kids?

All heterozygous for the defect

5-year-old boy loses spleen in an accident, give consultation?

Check for routine immunizations: DPT, poliovirus, and boosters.

MMR vaccine

Hib Vaccine (*H. Influenzae* type b)

Pneumovax (Vaccine using pneumococcal polysaccharides)

Daily low dose prophylactic antibiotics, higher doses when dental work is done or in case of any invasive surgical procedure.