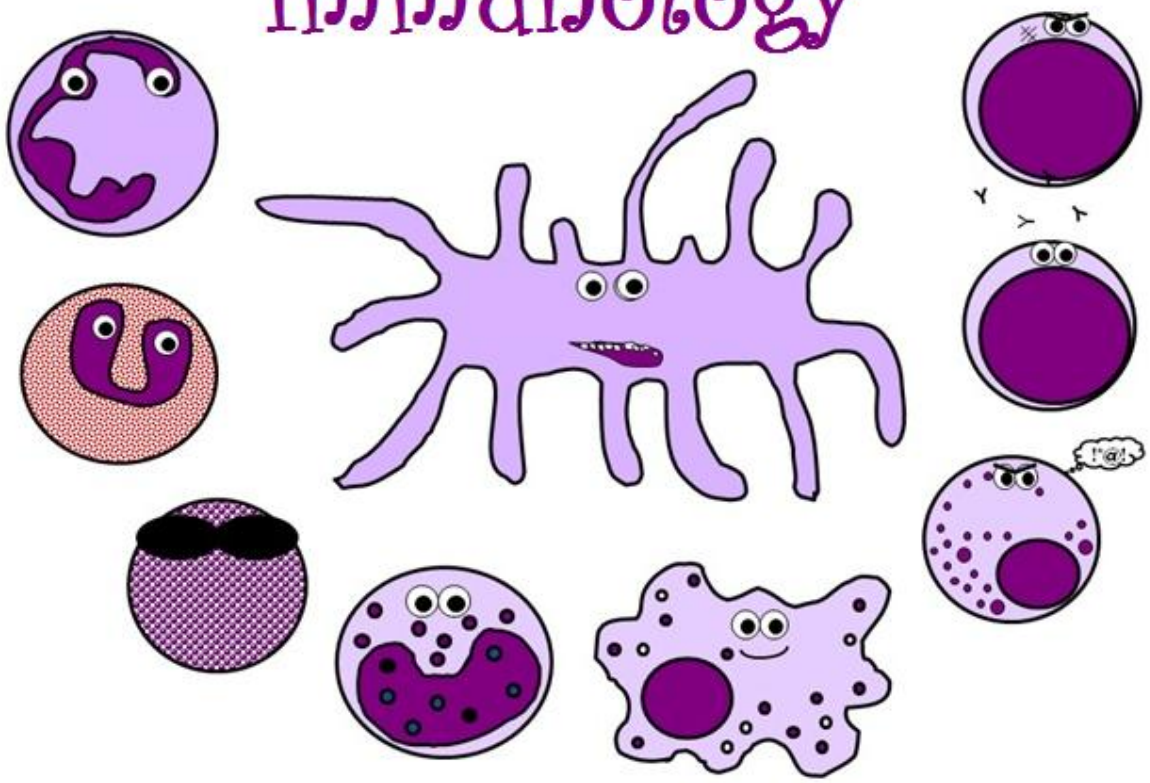
Immunology



Sheet Slides

Number:

Subject: Omenn Syndrome

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By the name of Allah the Compassionate the Merciful

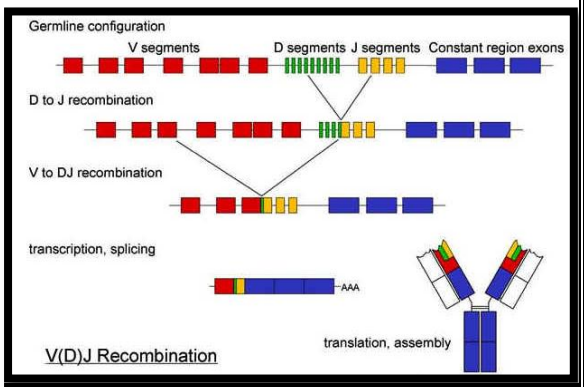
- In this sheet we will discuss the **Omenn Syndrome .**
- in this sheet I try to explain the case in my own way by combine what is explained in the lecture with what's written in the book , I do my best to **cover each piece of information that is mentioned in the book** ,it'll be really enough to read this sheet without going back to the book bcz I just reformulate the information in another scenario to make it easier !

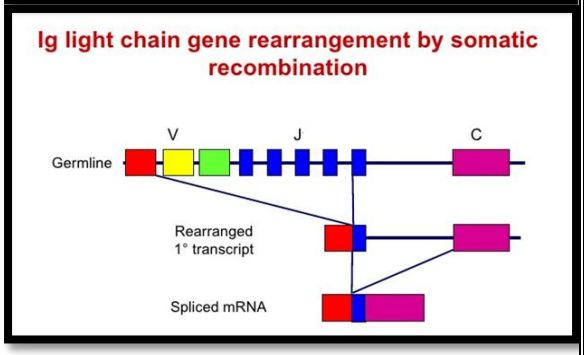
Omenn Syndrome

➢ **The recombination process :**

- **B cells** are synthesized and become immunocompetent in the **Bone Marrow** but **T cells** are synthesized in the **Bone Marrow** and become immunocompetent in the **Thymus** .

- Once the B cells are activated then they will proliferate, some of them will be plasma cells others B memory. Plasma cells are responsible for Antibody production. Antibody structure is composed of **two heavy chain and two light chain**





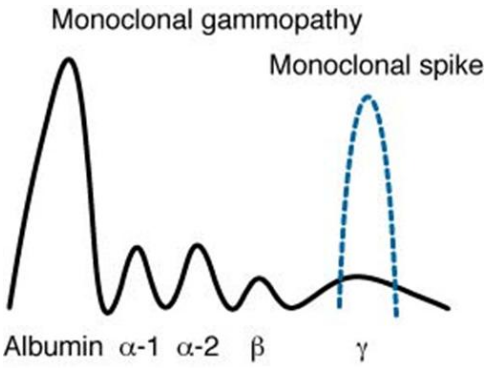
  - ✓ The chromosome that contains genes of the heavy chain has **segments V (variability) , D (diversity) , J (joining) and C (constant) .**

  - ✓ the chromosome that contains genes of the light chain has **segments V (variability) , J (joining) and C (constant) .{{ NO D segment }} .**

  - ✓ both heavy and light chains have **variable and constant regions** , ***constant*** *region of the* ***heavy chain*** determine the type of the immunoglobulin **{{ IgM/IgA/IgG/IgD/ IgE}}**

but *the **constant** region of the **light** chain* determine whether it's **lambda or kappa** light chains ; in our bodies their percentage in normal situation 60% >> kappa and 40% >> lambda .

- ✓ patients with **multiple myeloma** , one clone of b cells will produce a lot of Abs from the same type that's why it's called **Monoclonal Gammopathy** .
  by doing electrophoresis , we can notice that there increase in the production of one clone as you see in the figure , what the doctor wanted to say **that we have to determine whether those Abs are lambda or kappa bcz the treatment is different !**



- ✓ the **same plasma cell** produces abs against one type of antigens and they are whether kappa r lambda , **cannot produce one abs that is kappa and another one that is lambda .**

- T cells have on their surfaces what are called TCR (T cell Receptor) those TCRs are composed of **two chains alpha and beta** .
  - ✓ **Beta** chain >>> chromosome has segments V (variability) , D (diversity) , J (joining) and C (constant) **. {{ Just like the heavy chain }}**
  - ✓ **alpha chain** >>> chromosome has segments V (variability) , J (joining) and C (constant) .
    {{ Just like the light chain }} .

*** The RAG1 and RAG2 are for both TCR and B cell immunoglobulin development.**

- **D and J gene segment are joined first**, followed by **joining of a V** gene segment to form VDJ.

- the recombination process is initiated by two **(RAG1& RAG 2)** which bind to a **specific area (RSS).**

- **RSS** (recombination signal sequences) the sequences where the enzymes will bind to we have two RSS :

  NOTE :
  heptamer is followed by the spacer then the nanomer.

  - ✓ **heptamer** (CACAGTG) where the **RAG2** will bind to .
  - ✓ **nanomer** (ACAAAAGTG) where **RAG1** will bind to.
  - ✓ If we change just one nucleotide in the RSS region >>> a problem in binding will result !
  - ✓ We can notice that there are a lot of similarities between heptamer and nanomer sequences ! " **من روح بعض".**

- In between these RSS , there is what is called a **spacer of 12 or 23 bases which will deleted** >>> those can be any sequence , their importance just to give space !

  - ✓ it's important to note that the **FIRST SPACER** in the **Kappa** chain >>> **12 bases** , in **the lambda chain** , it's **23 bases** and **Heavy chain >>> 23 bases . NOTE WE SAID THE FIRST SPACER !!!!**

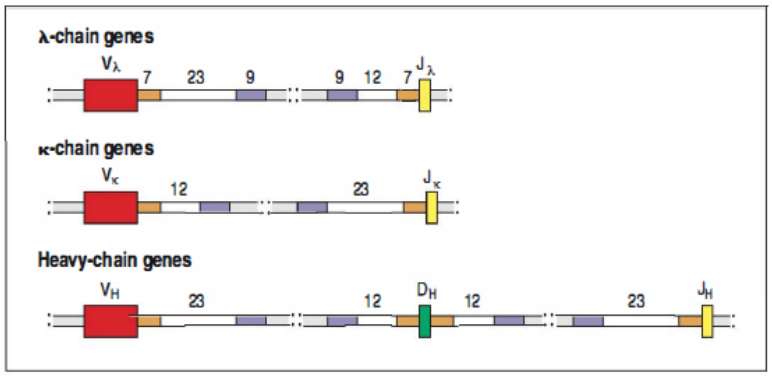


Fig. 7.2 Each V, D, or J gene segment is flanked by recombination signal sequences (RSSs). This is illustrated here with respect to the immunoglobulin genes. There are two types of RSS. One consists of a nonamer (9 nucleotides, shown in purple) and a heptamer (7 nucleotides, shown in orange) separated by a spacer of 12 nucleotides (white). The other consists of the same 9- and 7-nucleotide sequences separated by a 23-nucleotide spacer (white).

- As simple as it's ,,, **RAG-1 binds to the nonamer element** followed by binding of **RAG-2 to the heptamer**. The **DNA sequence** that forms the border between the heptamer and the coding segment is then **nicked**, and **a break in the double-stranded DNA occurs.** The coding ends are initially sealed by a **hairpin.**
  A series of ubiquitously expressed proteins (Ku70, Ku80, DNA-PKcs,

Artemis, DNA ligase IV (LIG4), XRCC4, and Cernunnos/XLF) are then recruited and mediate DNA repair and rejoining of coding and signal ends

- the recombination process is initiated by two enzymes **(RAG1& RAG 2)** which bind to a **specific area (RSS)** >>>
  when there is **a complete loss for RAG1 and RAG2** that will lead to **Severe Combined Immune Deficiency (SCID)** patients die quickly bcz the development of B cells and T cells is completely abolished but when there is a **partial problem as missense mutation** that ends up with **Omenn Syndrome** bcz this missense mutation change the codon >>> another amino acid is produced >>> **affects the activity of these enzymes {{ in our case it's decreased into 20% of normal activity }} .**

- **Missense mutation :** is a **point mutation** in which a single nucleotide change results in a codon that codes **for a different amino acid** . It is a type of nonsynonymous substitution .

- Again
  If either of the RAG genes is knocked out *by homologous recombination in* ***Mice*** *,* ***the development of B cells and T cells is completely abolished*** *and the mice have severe combined immunodeficiency.*
  Mutations in RAGl and RAG2have also been found in cases ***of human SCID*** *with lack of both T and B cellsCr-B- SCID).*
  In addition, ***defects of Artemis, LIG4, and DNA-PK have been also identified in patients with r-B- SCID, and mutations of Cernunnos/XLFcause combined immunodeficiency with markedly reduced numbers of T and B lymphocytes.***
  However, ***hypomorphic mutations*** *in these genes may allow* ***residual protein expression and function and may result in a different phenotype*** *,in which autoimmune* ***manifestations associate with severe immunodeficiency****. Omenn syndrome is the prototype of these conditions, and is most often due to* ***missense mutations in the RAG genes.***

- This video for a quick review of recombination process to be able to understand this related clinical case , https://www.youtube.com/watch?v=chwXp564-6o

The figure in the next page from the book to summarize the recombination process !

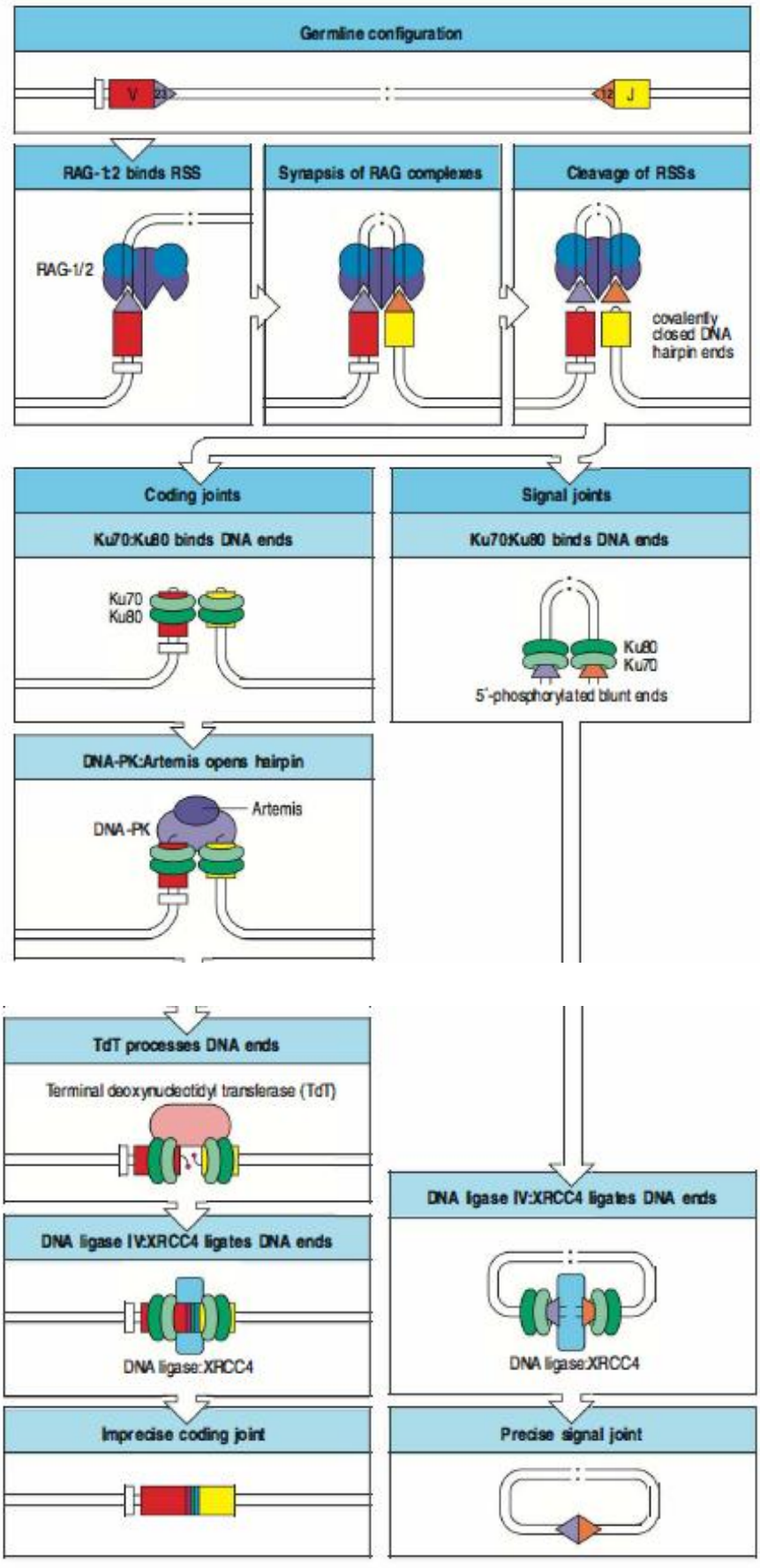


Fig. 7.3 Steps in the V(D)J recombination process. V(D)J recombination is initiated by the lymphocyte-specific RAG-1 and RAG-2 enzymes, which recognize the recombination signal sequences (RSSs) that flank the coding variable (V), diversity (D), and joining (J) elements. The DNA double-strand break at the coding ends is initially sealed by a hairpin. In the second step of the process, the ubiquitously expressed Ku70 and Ku80 are recruited both at coding ends and at signal ends. DNA-protein kinase catalytic subunit (DNA-PKcs) and Artemis are also recruited to the coding ends, and Artemis mediates opening of the coding-end hairpins. The enzyme terminal nucleotide transferase (TdT) may introduce additional nucleotides at the junction between coding elements. Finally, the enzymes DNA ligase IV and XRCC4 (involved in DNA repair and ligation) are recruited at both coding and signal ends and mediate the formation of coding and signal joints. Another enzyme, Cernunnos/XLF (not shown in the figure), also participates in the DNA repair process.

The case of Ricardo Reis: a bright red rash betrays an immunodeficiency.

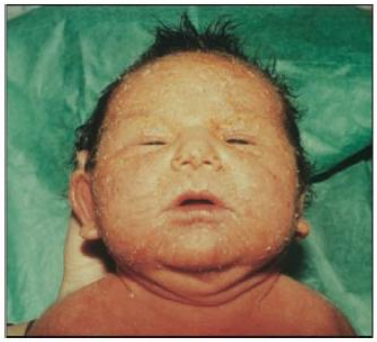
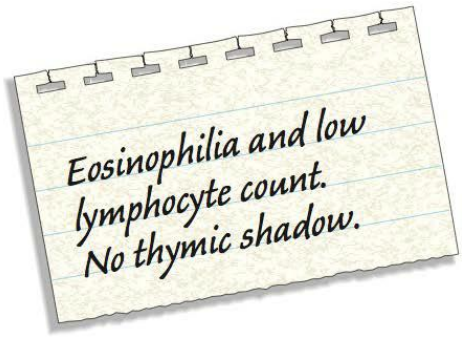


Fig. 7.4 Bright scaly red rash on the face and shoulders of an infant with Omenn syndrome.

- **History :**
  - ✓ **At birth**, Ricardo seemed to be **a normal healthy baby**. He gained **weight normally** and cried vigorously.
  - ✓ Soon after birth, his mother noticed that he had **10 loose bowel movements a day.**
  - ✓ **17th day after birth**, he developed **a rash on his legs**, which over the **next 7 days spread over his entire body**.
  - ✓ His parents brought him to the **emergency room** at Children's Hospital, and also reported that he had had **a dry cough for the past week**.
  - ✓ Ricardo's parents had three normal children, but had had **two other children, a boy and a girl, who had died soon after the onset of a similar rash at 1 month old.**
  - ✓ The parents **were first cousins** of Portuguese extraction.

- **physical examination:**
  - ✓ Ricardo's weight, length, and head circumference were **normal.**
  - ✓ The diffuse **papular scaly rash was worst on his face but also covered his trunk and extremities**
  - ✓ Small **blisters were present on his palms and the soles of his feet** , which were **red**.
  - ✓ **purulent conjunctivitis {yellow discharge from his eyes}.**
  - ✓ eardrums were normal,
  - ✓ no lymph nodes could be felt,
  - ✓ heart and lungs were normal.
  - ✓ The liver and spleen were not enlarged.

- After we took a full history from our patient and did a full physical examination , we decided to **do CBC** , the results were :
  - ✓ hemoglobin was 8.4 g dl-1 **(low)**
  - ✓ platelet count was 460,000 (**slightly elevated**)



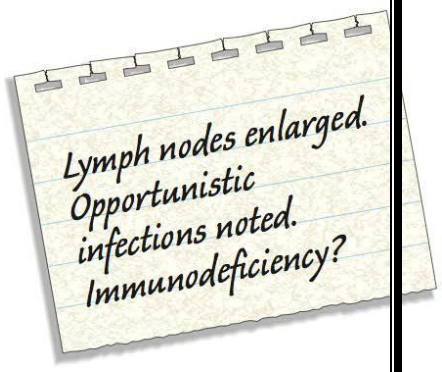
- ✓ white blood cell count was 8000 cells /ml (normal)
  >>> we said in pathology that we **have to see the percentage of each type** :
  56% were eosinophils (it's high ; the normal percentage 1-6% >>> **eosinophilia why ?! bcz of T-helper 2 as will be explained later in the sheet**)
  23% monocytes (**high** , the normal percentage 2-10%)
  15% neutrophils (low , the normal percentage 40-75% >>> **neutropenia)**
  6% lymphocytes (low ; normal percentage 20-45% >>> **lymphopenia)**

- After that , we decided to do **a BM examination** >>> preponderance of eosinophil precursors .
- Then we measure the level of each Ig in his serum to find that :
  lgG level was 55 mg dl-1 (**low** ;normal 400 mg dl-1)
  lgA and lgM were **undetectable**
  lgE was 7200 IU ml-1 **(very high** ; normal <50 IU ml-1).WHY?! that will be explained later in the sheet ☺

- A **skin biopsy** showed that the dermis was infiltrated with large numbers of **eosinophils, lymphocytes, and macrophages (inflammatory cells) . Large numbers of cells surrounded the blood vessels.>>> that what lead to erythema (red rash).**

- An **X-ray** of chest showed clear lungs and a normal cardiac shadow; there was **no thymic shadow!**

- In the hospital, Ricardo's condition rapidly worsened !! ☹ .
  He developed **enlarged lymph nodes in the neck and groin**, **and pus accumulated in the skin behind his ear.** This was drained, and **Staphylococcus aureus and Candida albicans were cultured from the drainage fluid**. **Thrush {Candida albicans)** was noticed in his mouth. These opportunistic infections give us indication of a drop in immunity!

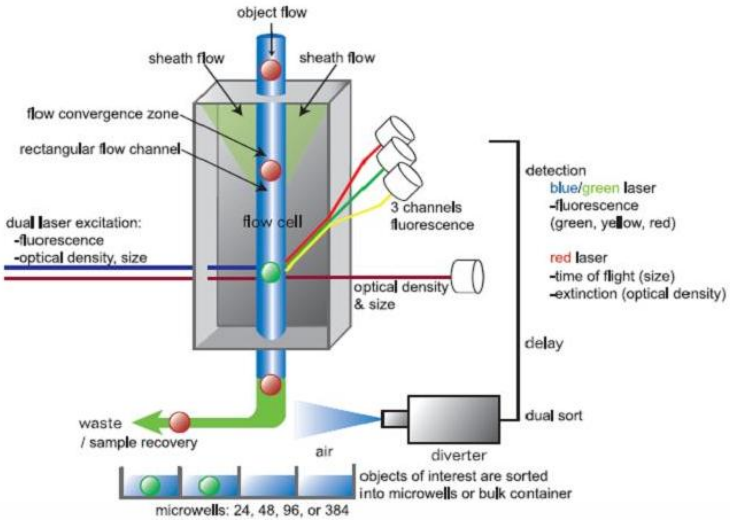


- It's Autosomal Recessive **{{ in case of Ricado , his mother and father were normal , a brother and a sister died bcz of this disease }} .**

- Then we consulted the immunologist who ordered **blood tests** that revealed **an absence of B cells and a paucity of T cells.**

  - ✓ From histology , we said we cannot differentiate between the B and T cells **{they have same morphology }** unless we do **Flow-cytometry analysis**.
  - ✓ Ricardo's peripheral blood lymphocytes responded poorly to stimulation with phytohemagglutinin and with anti-CD3 monoclonal antibody.

  - ✓ **Flow-cytometry analysis**:
    a laser- or impedance-based, biophysical technology employed **in cell counting, cell sorting, biomarker detection and protein engineering,** by suspending cells in a stream of fluid and **passing them one by one by an electronic detection apparatus** ,,,
    we already treated those cells with abs with flourcsent lights ,for example **B cell express CD19 / CD20 and T cells express CD2/CD3/CD5** and that we are looking for .



    so here we treated our cells with **anti- CD19 for** example if **the reaction occurs then it's B cell** .

  - ✓ In On FACS analysis:
    - ➢ **no cells were found that reacted with anti-CD19, which detects B cells** . ((that means there **are no detectable B cells**)).

    - ➢ **Eighty percent of the lymphocytes were CD4+, and 15% were CDS+.** Flow-cytometry analysis of Ricardo's peripheral T lymphocytes, <u>using monoclonal antibodies directed against various families of T-cell receptor Va and Vp sequences</u> showed **that only few of them were expressed**, indicating **an oligoclonal T-cell receptor repertoire { the diversity of TCR and their ability to**

recognize the antigen is very low} .

- ✓ **All the lymphocytes were CD3+,** of which 90% coexpressed the activation marker CD4
- ✓ 5RO, **and 65% expressed major histocompatibility complex (MHC) class II molecules, another marker of T-cell activation**

- Then , RAG1 and RAG2 genes were sequenced, and homozygosity for the Arg222Gin {R229Q) **missense mutation was found in the RAG2 gene**. The T cells were definitively identified as Ricardo's (and not as transferred maternal T cells) by HLA typing.

- While these studies were being carried out**, Ricardo developed Pneumocystis jirovecii pneumonia (pneumocystis carinii)** and died of respiratory failure :'(
- It's important to know that pneumocystis carinii is an **opportunistic** infection that occurs only on there is a **drop in the immunity** such as **AIDS** patients ((AIDS is discovered bcz patients are infected by opportunistic infections as pneumocystis carinii then they discovered the AIDS)) .

- When the patient comes with **opportunistic infections (pneumocystis carinii , Candida infections)** we should be careful ; if he's **a baby** we think of **primary** immune deficiency but if he's **adult** we think of **secondary** immune deficiency like AIDS .

- **The RAG enzymes essential for V(D)J recombination** were first discovered in mice and later identified in humans**. Infants with the autosomal recessive** form of **severe combined immunodeficiency (SCID)** were screened for mutations in these genes**, and several cases were identified in which RAG-1 or RAG-2 was deficient**. These infants **lacked T and B lymphocytes, but had a normal number of NK cells; hence they had r-B-NK+ SCID.**

- Some patients were found with **missense mutations in the RAG genes such that only partial enzyme activity was expresse**d. An examination of patients with a form of SCID **called Omenn syndrome revealed further missense mutations in RAG genes.**

- This syndrome is characterized by :
  i. **early onset of a generalized red rash (erythroderma) ((** activated T cells have **homing receptors { chemokine receptors }** that actually can go to the skin then secret their own chemokines , these chemokines will **recruit the inflammatory cells like the monocytes and eosinophils** , they will **cause perivascular inflammation >> vasodilatation** which leads to **redness rash and edema** < curly skin in the groin and legs bcz of Edema)

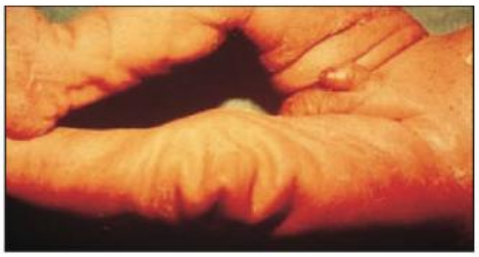


Fig. 7.5 Legs and groin of an infant with Omenn syndrome. The skin is bright red and wrinkled from edema and the infiltration of inflammatory cells.

  ii. failure to thrive.
  iii. protracted diarrhea
  iv. enlargement of the liver, spleen, and lymph nodes { the activated T cells will proliferate then that will leads to enlargement }.
  v. A high eosinophil count (eosinophilia) is usually encountered, together with a lack of B lymphocytes and a marked decrease in T cells.
  vi. Immunoglobulins are also markedly decreased, but IgE levels are raised.
    - **NOTE** : the reason why there is increase in the IgE despite the fact that there is absence of the B cells in blood test :
      1. we should be careful there **are no detectable B cells** but there are some B cells we cannot detect in our test !
      2. when we talked about **a class switching** in B cell maturation , we said that the switching into **IgE** is triggered by cytokines { **IL-4 and IL-5** } that are secreted by **T-helper 2 cells** .

***T-helper polymerization :**
according to the **environment** , t-helper will be whether T-helper1 and T-helper2 .

** **T-helper 1** >>> secret **pro-inflammatory cytokines** such **as INF-** γ ; they trigger inflammation to fight cancer ,infection ,, .

** **T-helper 2** >>> secret **anti-inflammatory cytokines** such as **IL-4/IL-5 /IL-10 /IL-13**.

*** some diseases are triggered by T-helper 1 others by T-helper 2 (such **asthma is triggered by T-helper 2**).

- What is really happening in our patient "Ricardo " >>> the **few T cells** work as T-**helper 2** which will **secret IL4 and IL5** then that will lead to **class switch into IgE** , also those interleukins will **recruit the eosinophils** and ends up with **eosinophilia**.
- Note : IgE is functional and it's the reason of the allergy that the patient suffers from !

- As only partial ability to execute V(D)J recombination is retained by the mutated enzyme, in most case**s no mature circulating B cells are detected and the few T cells that are found are oligoclonal**; that is, they are the products of a *limited number of different clones*. **These oligoclonal T cells infiltrate and cause significant damage in target organs.**

- **How does the RAG function assist !?**
  - ✓ We use J beta probe then Southern blot for DNA sequencing ((bad bcz they use radiation)) .
  - ✓ Northern blotting >> RNA sequencing.
  - ✓ Western blotting >> protein sequencing .

  * ***To assist the activity for RAG enzymes :***

  by using **viral vector** , there are sites called **multiple cloning sites** at which **we can cut and put the genes** we want to study .
  in this case we **put genes of RAG that are mutant** and in another vector we **put RAG genes that are functioning normally.**
  then put these vectors in fibroblasts then these vectors enter these fibroblast under our control , **once they enter those cells start to produce RAG enzymes .**
  then **we take these enzymes and put them with DNA sequences** (we designed and labeled them) when these DNA sequences are cut and the recombination process occurs , a special fluorescent color is appeared then we compare the normal RAG and the mutant one according to the fluorescent colors !
  In Ricardo case >>> 20% .

- As illustrated by this case**, Omenn syndrome is usually rapidly fatal** ☹ unless it **is treated by bone marrow transplantation**, which may result **in full correction of the disease**.

- As with the RAG genes, **Omenn syndrome** occurs when the defect **is 'leaky'**; that is, due to **a missense mutation** that **severely impairs** but **does not abolish function,** allowing **a few T cells to develop**.

- This part is not explained in the lecture but it's explained in the book so we have to study it !

  ** It is most likely that in Omenn syndrome the **autoimmune manifestations**, with **infiltration of target organs by oligoclonal T cells**,

reflect several mechanisms, as demonstrated by studies in patients and in animal models of the disease:

- ✓ **Poor generation of T lymphocytes in the thymus** results in **impaired maturation of medullary thymic epithelial cells** and **reduced expression of AIRE**, thus impinging on **the deletion of self-reactive T cells**.
- ✓ Furthermore, **generation of regulatory T cells in the thymus** is also impaired, **affecting peripheral tolerance** .
- ✓ Finally, the few T cells that are generated in the thymus of patients with Omenn syndrome undergo **extensive peripheral expansion (homeostatic proliferation) and secrete increased amounts of cytokines**, including **inflammatory (IFN-y) and TH2 (IL-4, IL-5) cytokines.**

** Apart from the lymphocyte-specific RAG proteins, V(D)J recombination also involves **proteins of the nonhomologous end-joining pathway** that are universally used for **DNA repair and recombination in human cells** . In addition to **r-B-NK+ SCID**, patients with defects in these genes (Artemis, DNA-PK, LIG4, and Cernunnos/XLF) **present increased cellular sensitivity to ionizing radiation**, because they **are unable to repair radiation-induced DNA damage**. These radiosensitive forms of T-B-NK+ SCID are often associated with **extraimmune clinical manifestations, such as microcephaly, neurodevelopmental problems, and growth and development defects.**

** Genetic defects that result in a severe, but incomplete, impairment of T-cell development by interfering with mechanisms other than V(D)J recombination can also result in Omenn syndrome. These include IL-7Ra chain deficiency (IL-7 is required for lymphocyte development), 'Yc deficiency (X-linked SCID), and mutations of the RMRP gene. The last of these causes cartilage hair hypoplasia, a condition characterized by dwarfism, sparse hair, a variable degree of immunodeficiency, and hematological abnormalities

➢ Questions.

1. How do you explain the high lgE level and eosinophilia in this patient?
2. How do you explain the enlargement of the lymph nodes in this patient?
3. How does Ricardo's family history help you determine the mode of inheritance of Omenn syndrome?

4. A bright red rash (erythroderma) is characteristic of Omenn syndrome. What causes this rash?

➢ Answers :

1. Answer 1

The few T cells produced and activated must have had a T H2 phenotype and secreted large amounts of interleukin-4 **(IL-4) and interleukin-5 (IL-5).** IL-4 is required for **switching to IgE** synthesis and IL-5 for **the recruitment of eosinophils. The few B cells in the patient** (which were below the limit of detection) must have been induced to switch immunoglobulin class to IgE.

2. Answer 2

The few clones of T cells that were able to mature were activated, as shown by their surface expression of **CD45RO and MHC class II molecules**. The activated clones would have **expanded within the lymph node.**

3. Answer 3

Neither of Ricardo's parents had Omenn syndrome, but Ricardo had **an affected brother and an affected sister**. This indicates **Mendelian autosomal recessive inheritance of the defec**t. If the defect had been *X -linked recessive, a female would not have been affected; if it were dominant, one of the parents would have had to have been affected.*

4. Answer 4

The T cells that are present are activated, as shown by their expression of **CD45RO and MHC class II molecules, and express homing receptors for the skin**. In the skin, the activated T cells secrete **chemokines** that attract other **inflammatory cells, such as monocytes and eosinophils,** into the skin. **The perivascular inflammation in the skin causes the blood vessels to dilate, and this appears as a bright red rash**.

➢ **To sum up plz watch this video**
https://www.youtube.com/watch?v=N0EV2OGFTK4

Wish you all best of luck ☺
sorry for any mistake ^^
Ayat M.Zghoul