Primary immunodeficiency disorders (PID) – 24th April 2020

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Slide2:

From Manolio TA et al. : Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio).

Every genetic variant could potentially lead to an abnormal protein expression or function.

The more frequent, the less likely this will cause a severe damage of the organism as it is shared by a broader population.

Slide 3:

Considering PID or inborn errors of immunity (IEI), these diseases are on the extreme left of the graph as rare or even private mutation can lead to serious genetic immune defect.

Slide 4:

Primary immunodeficiency disorders (PID) are **monogenic diseases of the immune system**. These affections give rise to complex diseases with a wide range of susceptibility to infections, from life-threatening severe combined immunodeficiency (SCID), with high risk of opportunistic microorganisms, to very specific deficiencies in susceptibility to particular infections, as seen with predisposition to develop herpes encephalitis in TLR3-deficient patients. From the innate to adaptive immune system every aspect of the immune response can be affected in PID. Moreover, PIDs encompass not only deficiencies in immune activation, but also deficiencies in immune regulation, which manifest with inappropriate excessive reactions with **autoimmune or autoinflammatory diseases (AID**).

Indeed, they are Rare Inherited Immunodeficiency, Usually, due to a single mutation giving rise to a Unique immune phenotype. Importantly, because the immune system can be explored in-depth through a blood draw, it is Easy to explore. Importantly, the first therapy success has been achieved in PID.

Slide 5:

All type of inheritance pattern is found in PID, with the classical autosomal dominance, autosomal recessive, X-linked but also de novo mutation can arise.

Importantly somatic mutation is not to be forgotten as they can be found, specifically in some proliferative disease or inflammation (such as in gene coding for FAS protein or in *NLRC4, NLRP3* genes).

Other parameters need to be considered. For example, the importance of the quantity of a normal protein as in some case only 50% of normal protein expression is not sufficient (as seen in *GATA2* deficiency), this situation is a called haploinsufficiency.

Finally, it is important to keep in mind that not all the mutations are severe and different mutations in the same gene can give rise to different phenotype as seen in hypomorphic mutation.

Finally known genes causing disease are exponentially discovered since next-generation sequencing (NGS), especially with the worldwide usage of whole exome sequencing (WES).

More than 400 unique genes causing diseases are known at the present time and there are probably another 2500 ones to be discovered.

Slide 6:

Previously PID subgroups were classified as related to the clinical presentation.

Patients with a T cell defect were presenting early on with opportunistic diseases, infections that are not present or less severe in people with a competent immune system. Failure to thrive is a very common feature in these patients. Importantly, they can present with a “graft-

versus host" disease (GVHD) at birth when T cells from maternal origin are present in their blood and react against their self-antigen, cells and organs.

Slide 7:

Patients with B cell defect are more likely to present with infection secondary to encapsulated bacteria as antibodies are crucial for neutralising bacteria through a mechanism called opsonisation. They can also develop viral infection specifically after live vaccine (cases of vaccine related polio after the live polio vaccine). Interestingly these patients are usually becoming symptomatic once the maternal immunoglobulins IgG, acquired passively in utero disappear, between 4-6 months after birth.

Slide 8:

Patients with a defect in myeloid cells will develop infection secondary to bacterial and fungal microorganisms as these cells are important to kill these pathogens through phagocytosis and oxidative burst. Typically, these patients will present with abscesses, in the skin, mucosa, lung, liver and additional formation of granulomas.

Slide 9:

Now, PID diagnosis is molecularly driven and, as previously mentioned, there are more than 430 genes identified. To my opinion, once someone is working in genetic diagnosis the most important is probably not to be an immunologist expert but probably to be able to use the most reliable source to identify the genetic diagnosis related to immune disorders. To help in this task, an international panel of experts is publishing every 2 years an update about all known molecular diagnosis related to PID. Here is the link to the article and I hope you will be able to consult it if you need it in a near future.

https://link.springer.com/article/10.1007/s10875-019-00737-x

Slide 10:

For this class I will explain in depth only the first part of the PID, on SCID, as this is considered as a diagnostic emergency to which any genetic clinical lab could be confronted. Recognising early on a SCID diagnosis is a real lifesaver, as left untreated or unrecognized the survival expectancy of these children is severely compromised.

I will leave the rest of the slides with other PID in the presentation as most of them are self-explained and will not add more specific details.

Immunology is not as complex as it seems, and especially if addressed using these 2 different following aspects. 1/ Cell development from one hematopoietic stem cells that divides and differentiates into several different subsets. 2./ Cellular – molecular function.

If one of these 2 aspects are missing, then PID can occur.

This slide is representing the immune cell development from one hematopoietic stem cells to all the possible different cell subsets. Any block in the cell development will subsequently provoke the absence of a specific cell subtype.

Slide 11:

Block in T cell development.

Slide 12:

SCID stands for Severe Combined ImmunoDeficiency and is encountered in all patients having a profound T cell defect. As explained in the slide 6, absence of T cells leads to susceptibility to severe opportunistic infection and chance to survival is compromised.

Originally young children affected by this disorder were called "bubble children" as the only chance to survive without encountering a pathogen was to live in a "sterile" environment.

To date, curative treatment for these children is existing such as hematopoietic stem cell transplantation or gene therapy when a matching donor is not available.

Slide 13:

Focussing back on the hematopoietic cell development and furthermore on the lymphoid compartment, we can further specifically look at the T cell development.

Slide 14:

Focussing on lymphocytes, NK cells are mainly generated in the bone marrow and are functioning once in the periphery. On the other side, T cells are exciting the bone as early progenitors and need to continue their development in the thymus. There, they mature and are "educated" to recognize self-antigen from non self-antigen. This is called "negative selection" where most of the self-reacting T cells are depleted. Once their maturation is achieved, they can go in the periphery as naïve cells and are fully functional.

B cells are maturing first in the bone marrow but needs also to finalise their maturation in the periphery, in the spleen. Any block in this differentiation step can be responsible for the lack of lymphocyte development and therefore causes a SCID phenotype.

Slide 15:

The most common mutation found in SCID is a mutation in a gene coding for **γC** protein, the common chain of different cytokine receptors (IL2R, IL4R, IL7R, IL9R, IL15R and IL21R). Lymphocytes are dependant of some cytokines for their survival. Without important cytokines, the cells cannot further differentiate and/or are dying.

T cells are really dependent on IL2 and IL7 and some of them on IL15. NK cells are mainly dependant on IL15. B cells survival are less dependent of these cytokines but B cells function is definitively not optimal without a competent T cell in the environment.

Therefore, the immunophenotype of these patients in the clinic will be a low lymphocyte count in the blood with complete absence of T cells and NK cells while B cells are detected.

We can call them as T-NK-B+ SCID. This gene is carried on the X chromosome, this is indeed an X-linked disease. If recognised on time patients can be cured with a bone marrow transplantation/ Hematopoietic stem cell transplantation (HSCT) and for some gene therapy is a valuable treatment.

Slide 16:

Mutation in ***JAK3*** will give rise the exact identical phenotype that is present in γC defect.

Indeed, JAK3 is the immediate downstream protein after cytokine receptor activation.

Importantly, this is an autosomal recessive gene defect, therefore girls can be affected.

HSCT is the only curative treatment for these patients as gene therapy is not available for this gene.

Slide 17:

IL7RA is the other chain that composes the IL7 receptor with the γC. Being one of the most important cytokines for T cells, only this subset will be affected. Patients will also have lymphopenia but characterized by T-NK+B+ in their blood. HSCT is the only curative treatment for these patients as gene therapy is not available for this gene.

Slide 18:

Another important SCID diagnosis can be attributed to a defect in ***ADA,*** gene coding for adenosine deaminase, an important enzyme to clear out nucleoside metabolites. In its absence adenosine metabolites are accumulating in the cells which is particularly toxic for all types of lymphocytes leading to cell apoptosis. Patients are then deeply lymphopenic in all cell subtypes as T-NK-B- SCID. Importantly as lymphocytes are not the only affected cells, patients are often presenting with additional features (neurological defect, bone dysplasia

and others). Interestingly, the first treatment proposed for these patients was additional enzyme replacement therapy, available since more than 30 year ago. However, this is not a curative treatment for which only HSCT and gene therapy have shown definitive long-term improvement.

Slide 19:

The 2 following molecules have the particularity to affect both T and B cells differentiation and function. And to make it more complex, it gives rise to a broader and variate phenotype according to the severity of the mutation.

Both molecules are involved in what is called the V-D-J rearrangement which is an important step to make functional TCR and BCR, meaning functional T and B cells. Our immune system is able to recognise many more antigens that our actual genome could support as a simple transcription of all different genes. To achieve the recognition of thousands of different antigens, a unique mechanism of genetic recombination occurs during the development of T and B lymphocytes in the TCR and BCR locus. Part of the genome is cut and a unique combination of the variable region recognising the antigen is formed allowing exponential possibility of unique antigen identification.

RAG1 and ***RAG2*** genes are playing an important role in this process as being one of the first enzymes involved. Indeed, RAG is acting as a "cutter" of the double stranded DNA to initiate the process of V-D-J rearrangement. In their absence, the recombination cannot occur and therefore no functional TCR or BCR are produced, preventing T and B cell maturation at an early stage. Patients are then diagnosed with a T-NK+B- phenotype. Here again the only curative treatment is a HSCT.

Interestingly, there is a broad clinical phenotype that can be present in patients affected with RAG mutation (cf **slide 21**). In some case, there is a partial defect in the enzyme activity and some degree or recombination can still occur, however, the quality of the T and B cells are usually impaired as for instance negative selection in the thymus is not optimal. Patients have then some T cells in periphery but some of them are autoreactive generating proliferation of autoreactive T cells with autoimmune manifestations. This is called "**Omenn Syndrome**".

The only curative treatment is here again HSCT.

Slide 20:

Defect in the protein **ARTEMIS (*DCLRE1C*** gene) is quite similar as the defect reported in RAG mutation. ARTEMIS acts downstream the V-D-J rearrangement process and act with a complex in the final step before the ligation of the final newly formed VDJ region.

The phenotype of ARTEMIS deficient patients is exactly similar than the one from the RAG deficient patients with the exception of ARTEMIS being a member of the "DNA repair" engineering tools. This means that these patients are susceptible to DNA damage and for instance, while receiving HSCT as a curative treatment they cannot receive any form of irradiation for their conditioning pre-transplant as it would be fatal to them.

Slide 21:

This slide indicates the broad spectrum of RAG/ARTEMIS deficiency phenotype, from SCID to milder phenotype (leaky SCID) in which immunodeficiency is less an issue than autoimmune manifestations generated by the presence of autoreactive clones of T and B cells in the periphery.

Slide 22:

There is additional TCR related defect with a wide range of clinical presentation.

Slide 23:

There is additional T cell defect that can be exclusively caused by a loss of function, with a presence of normal lymphocyte count. Different defects either in CD4 or CD8 defect are included in this group. I however chose to illustrate only one of them as it is causing a fulminant inflammatory syndrome due to cytokine storm, a mechanism different but sharing some similar features than what we encounter during these difficult moments while fighting against the coronavirus.

Slide 24:

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening systemic inflammatory disorder. This inflammation is characterized by an excessive activation of the immune system (mainly cytotoxic T cells and macrophages) and a cytokine storm (including IFNγ, IL6 and TNFα). Clinical manifestation includes prolonged fever, cytopenia, hepatosplenomegaly, hepatitis, and, in some patients, neurological symptoms. Classical presentation occurs after contact with a trigger such as a viral infection and can lead to the patient death by uncontrolled inflammation, multiple organ failure or secondary severe infection if the disease is left untreated.

Slide 25:

HLH is a syndrome which can be caused by several different conditions. Primary HLH describes patients with defects in genes related to **the cytotoxic pathways** of T/NK cells such as *PFR1, UNC13D, STX11, STXBP2, RAB27A, LYST* and *AP3B1* (in the latter three HLH is accompanied by albinism and dysfunction in neutrophils and platelets). In addition, defects in genes involved in EBV clearance (such as *SH2D1A* and *BIRC4*) will trigger HLH once affected patients will encounter EBV. These primary HLH are typically diagnosed in very young patients below one year of age. In HLH, patients battle against a severe inflammatory disease that requires drastic treatment such as steroids, etoposide and immunosuppressive reagents. HSCT is the only curative treatment.

Slide 26:

The standard model for HLH disease progression is one where the defect in cytotoxicity results in an inability to clear antigen presentation cells at the end of an infection, leading to extended synapse contact and increased activation of $CD8^+$ T cell. These $CD8^+$ T cells then have amplified expression of cytokines, most importantly IFNγ, which further activate the immune system, especially macrophages, creating a cytokine storm and critical inflammation.

Several additional immune defects in the T cell compartment have been described but won't be detailed during this presentation.

Slide 27: B cell defects.

Slide 28:

B cell maturation starts in the bone marrow and is achieved in the spleen giving rise to a competent mature and naïve B cells. Similar to T cells, different block in the B cell development will subsequently lead to the absence of mature B cells.

Slide 29:

X-linked agammaglobulinemia is the prototypical B cell defect associated with an absence of B cells and antibodies. The most common form is attributed to a defect in **BTK,** Bruton tyrosine kinase, a molecule right downstream the BCR. Lack of BTK abrogates any downstream signalling inhibiting B cells development, activation and survival (**slide 30**). Typical antibody deficiency is treated by antibody replacement therapy such as intravenous or subcutaneous immunoglobulin infusion.

Slide 31:

Another mutation that causes inadequate B cell response is the inability for B cells to receive the appropriate signal from T cells to perform their last step of maturation after having encounter an antigen. This is the result of defect in either ***CD40*** or ***CD40LG*** genes. This defect is preventing the B cells to switch their antibody to be either more performant by class switching (IgM to IgG) or by increasing their affinity against their recognized antigens (somatic hypermutation).

Slide 32:

Finally, this slide represents the most common and probably the least described B cell immune defect named as **common variable immunodeficiency (CVID).**

Slide 33:

The myeloid compartment is not spared from immune dysfunction.

Slide 34:

The myeloid cells can also experience a block during development and differentiation as expose in the following slide.

Slide 35:

Severe congenital neutropenias (SCN) comprise a genetically heterogeneous group of inborn errors of immunity, characterized by a differentiation arrest of granulopoiesis at the promyelocyte stage. The low absolute number of circulating mature neutrophils predisposes to life-threatening and recurrent infections from early childhood, including otitis, skin infections, deep abscesses, gingivitis, and septicemia. Furthermore, patients are at increased risk for the development of myelodysplastic syndromes or acute myeloid leukemia. Autosomal dominant (AD) mutations in ***ELANE***, encoding for neutrophil elastase (NE) and autosomal recessive mutations in ***HAX1***, are the most common cause of SCN, with mutations in 22 additional genes accounting for the remainder of the characterized cases.

The most common treatment is supportive with the administration of G-CSF that in most of the cases enable the neutrophils to mature to the periphery.

Slide 36 and 37:

The most common myeloid defect is probably found in the **chronic granulomatous disease (CGD)** secondary to an oxidative burst defect and leading to defective function of the phagocytes. Patients will present with recurrent abscesses, bacterial and fungal infection and granulomas. Five different proteins have been identified as causative of the disease (**gp91phox, X-linked (75%) and p22phox, p47phox, p67phox and p40phox**(AR)). Treatment for these patients are either supportive for milder cases but HSCT and gene therapy are proposed for the more severe cases.

Slide 39 and after are treating over gene therapy for PID and most of them are self-explained.