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TITLE: Primary T cell Immunodeficiencies

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Slide 1:

Hello, my name is Amir A. Sadighi Akha. I am a Consultant in the Department of Laboratory Medicine and Pathology at Mayo Clinic. Welcome to this Pearl of Laboratory Medicine on "Primary T cell Immunodeficiencies".

Slide 2:

My aim here is to provide a broad overview of primary T cell immunodeficiencies and the laboratory approach to their diagnosis and potential treatment, with particular emphasis on Severe Combined Immunodeficiency (SCID).

Let's start by defining primary immunodeficiencies (PIDs). This group of diseases is due to germline mutations of genes that affect the development and/or function of immune cells. This is in contrast to secondary immunodeficiencies that are the consequence of another disease, environmental factor(s) or certain modes of therapy.

The US rare diseases act of 2002 defines PIDs as rare diseases. This is because they affect fewer than 200,000 people or approximately 1 in 1500 individuals. While it may seem redundant, I think it is important to note here that as a group PIDs are far less prevalent than secondary immunodeficiencies.

There are different approaches to classifying PIDs (See Bousfiha et al., 2020, and Tangye et al. 2020, for the most recent IUIS classification of PIDs). Here, they are classified on the basis of the affected arm(s) of the immune system and/or the underlying mechanism of the observed defect(s). Clinically, PIDs can have a range of manifestations, from asymptomatic to those associated with a combination of severe infections, autoimmunity and malignancy.

Slide 3:

Our understanding of PIDs has undergone major developments within the past 25 years. One significant development is the increase in the number of existing entities and underlying molecular defects that have been identified. Identifying the underlying mutations for the PIDs has also shown that the clinical phenotypes associated with many entities are broader than first realised. The range of manifestations observed in RAG-1 and RAG-2 deficiencies are clear cases in point. Another major development is a conceptual shift in the definition of PIDs which now includes both the classical PIDs, exemplified by SCID and X-linked Agammaglobulinaemia (XLA), and PIDs with narrow phenotype, such as Mendelian Susceptibility to Mycobacterial Disease (MSMD). This concept is covered in depth in 2 papers by Casanova (Casanova, 2015a and Casanova, 2015b). Finally, I should mention the discovery of antibody-mediated phenocopies of PID, condition(s) where an autoantibody mimics a recognised PID's presentation in the absence of the underlying genetic basis for that PID. This subject is comprehensively covered by Sarah Browne in her review of the subject (Browne, 2014).

Slide 4:

Primary T cell immunodeficiencies are a group of genetic disorders that affect the development, activation and survival of T cells. They can affect T cells in isolation or in conjunction with other cell types, including B cells, NK cells and components of the innate immune system. They can also be a component of a broader clinical syndrome. The different entities can present at different time points in an individual's life, with some manifesting in early infancy and others first presenting themselves during adulthood. As is the case for primary immunodeficiencies in general, T cell immunodeficiencies can have a range of manifestations, from life-threatening susceptibility to infection to presenting mainly with autoimmune symptoms and/or susceptibility to neoplasia.

Slide 5:

There is not a single way to classify primary T cell immunodeficiencies. However, a common approach is to place these defects into 4 categories: 1) Defects in thymus organogenesis, 2) Typical SCID, 3) Atypical SCID, and 4) Combined Immunodeficiencies (CID). This slide lists the main subgroups in each of these categories. A comprehensive classification and discussion of these diseases can be found in the following references: (Bousfiha et al.2020; Roifman, 2019; Tangye et al., 2020).

Slide 6:

The two figures here highlight the underlying cause for a number of important T cell immunodeficiencies. They do so in the context of the main maturation steps in the thymus and the signalling pathways involved in T cell development and function.

The figure on the left illustrates the arrival of haematopoietic stem cells (HSC), and their sequential maturation through double-negative (DN) (CD4-CD8), double-positive (DP) (CD4+CD8+), and single-positive (CD4+) or (CD8+) stages in the thymus. The figure on the right displays the signalling cascades that are involved in the T cell response to a peptide presented by an antigen presenting cell (APC).

Defective thymus organogenesis would jeopardise T cell maturation, as can be the case in DiGeorge syndrome. Each of the deficiencies portrayed in these figures interferes with a particular stage: mutations in *AK2*, *ADA* and *PNP* fall under apoptosis of HSC and CLP (common lymphoid progenitor); the ones in γc (*IL2RG*), *JAK3* and *IL7RA* cause defective cytokine-dependent signalling; those in *RAG1/RAG2*, *DCLRE1C* (Artemis), *PRKDC* (DNA-PKc), *LIG IV,* and Cernunnos lead to defective V(D)J rearrangement; the ones affecting *CD45, CD3D (CD3δ), CD3E (CD3ε), CD3Z (CD3ζ), LCK* and *ZAP70* interfere with signalling through the T cell receptor (TCR). Mutations in *TAP* lead to MHC class I deficiency and those in *CIITA, RFX5, RFXAP* and *RFXANK* cause MHC class II deficiency, leading to defective maturation of CD8+ and CD4+ T cells respectively.

Slide 7:

In principle, one should adopt a tiered approach, in tandem with the patient's clinical presentation, when choosing laboratory tests to diagnose T cell immunodeficiencies. A simple CBC and differential can spot the decrease or absence of lymphocytes, which can be followed up by T, B and NK cell enumeration by flow cytometry. If necessary, extra markers can be used to determine the relative distribution of naïve T cells (CD45RA+) and the fraction of recent thymic emigrants (CD45RA+CD31+) within them, as well as memory T cells (CD45RO+) and their central, effector and T_{EMRA} subsets (Larbi and Fulop, 2014).

In addition to establishing the number and type of T cells in the patient, it may be necessary to evaluate their function. Lymphocyte proliferation assays can determine the capacity of the patient's T cells to proliferate in response to different stimulants. Each group of stimulants answers a particular question: Stimulation with PHA bypasses the T cell receptor and examines the cell's ability to divide. Using anti-CD3 will determine if the T cell receptor-mediated signalling is intact and can lead to proliferation, whereas the use of an antigen will clarify if the T cell can interact properly with an APC and divide in response to a protein it has previously encountered.

From a global perspective, performing the TREC assay is a third-tier test. In this context, a patient suspected of SCID based on clinical presentation or family history will be assessed for thymic function with the TREC assay in the stepwise approach depicted in this slide. However, as far as United States is concerned, baseline TREC analysis is part and parcel of newborn screening. It is expected that an increasing number of countries move towards this approach based on their respective national health policies and financial means.

Genetic testing can provide diagnostic confirmation of a particular diagnosis as well as a tool for family counselling. In the case of SCID, specifying the molecular cause can have therapeutic implications. For instance, the pre-transplant conditioning in cases of SCID with radio-sensitivity will be different with the ones that do not have such a proclivity.

Assessment of the TCRVβ repertoire is an important component of diagnosing a subset of T cell immunodeficiencies including Omenn syndrome and atypical DiGeorge. It is also useful in post-HSCT monitoring. This can be done by evaluating TCRVβ CDR3 (complementarity determining region 3) diversity by PCR. Normally, the Vβ families are polyclonal (more than 5 independent peaks) and the majority of them have a Gaussian distribution. By contrast, patients with Omenn can have oligoclonality (fewer than 5 independent peaks) in numerous Vβ families.

Slide 8:

Because of the importance of SCID, both in diagnostic and therapeutic terms, I'll mainly focus on this entity for the rest of this presentation. I'll then finish by highlighting the laboratory features of combined immunodeficiencies with defective T cell signalling.

SCID refers to a diverse group of genetic disorders, with 20 known and a number of unknown causes, that affect the adaptive immune system.

SCID patients have very low or absent number of T cells and lack adequate T cell function for survival, as well as absent or non-functional B cells. Generally, SCID patients seem healthy at birth but within a short period can present with severe opportunistic infections such as CMV infection, *Pneumocytis jiroveci* pneumonia, as well as chronic diarrhoea and failure to thrive. Due to the lack of B cell function, they are also susceptible to bacterial infections typically seen in patients with XLA: *Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus*. Unlike SCID patients, those with XLA are not susceptible to opportunistic pathogens.

SCID subtypes can be identified on the basis of autosomal recessive versus X-linked inheritance, the presence or absence of B cells and NK cells in the patient and the nature of the mutation, as tabulated here.

Prior to the use of haematopoietic stem cell transplantation (HSCT), SCID was universally fatal at an early age. Therefore, it should be treated as a medical emergency. The importance of newborn screening using TREC should be understood in this context, where the aim is to identify affected patients and to move them towards HSCT as soon as possible. The reason for the urgency is that the chance of a successful HSCT decreases with age, with the highest level of success achieved in infants under the age of 3 months. It has been established that the age of the recipient and the number of infectious episodes are two independent risk factors for the success of HSCT.

Although currently HSCT is the most prevalent form of treatment for SCID, there are other treatment options for a subset of SCID patients. These include enzyme replacement therapy for

ADA deficiency, and gene therapy for IL2RG and ADA deficiencies. In contrast to SCID, the proper treatment for defects in thymus organogenesis such as complete DiGeorge syndrome and Foxn1 deficiency is thymus transplant, not HSCT.

Slide 9:

This slide is based on the diagnostic criteria established by the Primary Immune Deficiency consortium. In addition to what is cited on the slide, I'd like to emphasise a few points. One is that the criteria established for lymphocyte proliferation to PHA refers to results obtained by the tritiated thymidine method and does not have a linear corollary to the findings by flow cytometry-based assays. Secondly, although initially Omenn syndrome was described in a subset of patients with RAG mutations, it is now clear that mutations in other genes including ILR7A, IL2RG, ADA, CHD7, RMRP and AK2 can also cause a similar phenotype.

Slide 10:

Before the implementation of newborn screening, suspicion of SCID depended on the emergence of clinical manifestations including infections with both ordinary and opportunistic organisms, chronic diarrhoea, failure to thrive, etc. and/or a family history of confirmed SCID in a sibling or another close relative.

The introduction of newborn screening using TREC has led to a sea change in the clinical approach to SCID. The rationale behind this test is that during the rearrangement of the TCRα locus (TCRA), TCRδ is excised creating *the* δ *REC-ψJa TREC* T cell receptor excision circle (TREC).

TRECs are present in the cell during T cell differentiation, but undergo progressive dilution as a result of T cell proliferation. In newborn screening, genomic DNA obtained from a dried blood spot collected at birth is used to enumerate TRECs as a measure of thymic activity. The assay is based on a qPCR method targeting the δ *REC-ψJa* signal joint region. Amplifying a reference gene, such as albumin, in parallel with TREC serves as the internal control. The DNA extraction method and the calibrator used can lead to variation in the TREC copy numbers reported by different laboratories. Lack of amplification in both the TREC and the reference gene can be due to insufficient DNA or the presence of inhibitors in the sample.

Very low or undetectable TREC numbers in the sample can be seen in infants with SCID or any other defect that undermines T cell development. Currently, all 50 states as well as the District of Columbia, Puerto Rico and the Navajo nation perform TREC assessment as part of newborn screening.

Slide 11:

So far, there have been two large scale reports on the use of newborn screening for SCID in the United States. The first of these was based on data from 10 States and the Navajo nation and included 3,030,083 newborn infants in total (Kwan et al., 2014). The second report, which overlaps with the first one, is based on the data obtained in California from 2010-2017 and includes 3,252,156 newborn infants (Amatuni et al., 2019).

The 2014 report identified 52 cases of SCID in the screened population (see the left side of the slide), therefore increasing the estimate for the incidence of SCID from 1:75000 to 1:58000. It also changed the perceived incidence of different genotypes. For example, the incidence of X-linked SCID in the screening programme was half of the previous estimates. As the rate of mutation in the IL2RG gene is constant, this indicated that the autosomal recessive causes of the disease have a higher incidence, which can be diagnosed on an equal footing with newborn screening. In addition, in certain instances, TREC uncovered unexpected aetiologies for the observed T cell lymphopaenia. The slide further tabulates the phenotype and genetic basis of the identified SCID cases.

The figure on the right provides a more granular assessment of the underlying cause and relative distribution of diseases that led to a low TREC in the 2014 report. It also depicts a practical algorithm for working up an abnormal TREC result in newborn screening.

Slide 12:

The newborn screening report from the State of California (2010-2017) (Amatuni et al., 2019) contains a similar number of infants as the 2014 study obtained from 10 States and the Navajo nation (Kwan et al., 2014). As pointed out earlier, there is overlap between the two studies as a fraction of the newborns from California are also included in the 2014 report. Despite demographic differences between the cohorts included in the two reports, the distribution of outcomes shows a high degree of similarity between these two studies.

Slides 13:

This slide shows the laboratory results obtained on a SCID patient at our laboratory. As you can see, it includes tests from all the tiers discussed in Slide 7. The patient has a

decrease in total T cell counts with a pronounced decrease in CD4+ T cells. The patient's TREC counts are below the limit of detection, pointing to impaired thymic output. One way to confirm this impairment is to look for recent thymic emigrants by flow cytometry, which by definition are naïve (express CD45RA) and CD31+. Unlike the control sample (middle panel), very few of the patient's cells (lower panel) express either CD45RA or CD31, independently confirming the TREC findings.

Stimulation with PHA was performed to examine the proliferative capacity of the patient's T cells. Very few of the patient's T cells proliferated after stimulation with PHA, indicating that even the cells present do not function properly. Finally, the patient's T cells have a more restricted TCRVβ repertoire diversity than expected under normal conditions. This in turn limits the spectrum of antigens that they can interact with and respond to.

Slides 14:

The tests that are used to identify patients with SCID can also be used to monitor the reconstitution of T cells by the thymus after HSCT. Here you can see the course of events over a 34-month period in a SCID patient who has undergone HSCT. The ordered tests include T cell enumeration, TREC and TCR Vβ spectratyping. Between February 2017 to November 2018, there is a gradual increase in T cell numbers and the TREC counts until they all fall into the normal reference range. There is a parallel increase in the diversity of TCR Vβ repertoire during this period. The follow-up testing in December 2019 is to make sure that the T cell reconstitution is sustained in the patient. Ordinarily, monitoring will continue over time, with the frequency determined by the outcome of each assessment.

Slide 15:

This slide depicts the molecular basis of genetic abnormalities of immunity in the context of T cell activation. The proteins in red are those that may cause primary defects in T cells but affect other cell types in a secondary manner. The ones in blue have cell-intrinsic functions in T cells as well as other cell types.

Obviously, discussing these defects in detail is far beyond the scope of this presentation. Instead, I'll emphasise the broad characteristics to look for in these diseases in the following slides.

Slide 16:

Signalling is important for different aspects of T cell function including cytokine production, effector mechanisms, immune regulation, proliferation and survival. Therefore, all these diseases can manifest simultaneously with both immune deficiency and immune dysregulation. Impaired T cell development and/or survival in the thymus and reduced effector function cause susceptibility to infection, whereas impaired negative selection of autoreactive T cells, impaired generation and/or function of Tregs and defective activation-induced cell death lead to immune dysregulation.

Slide 17:

From a laboratory perspective, different signalling defects will show varying degrees of T cell lymphopaenia, decreased number of naïve T cells and a change in the distribution of T cell subsets with a potential increase in effector memory T cells and T_{EMRA}. It is unclear if the latter is the result of persistent viral infections or a direct corollary of these diseases. At the humoral level, the full spectrum of immunoglobulin levels, from hypo- to hyper-gammaglobulinaemia have been observed. In addition, autoantibodies can be present in certain instances.

The combination of the laboratory results obtained in each case, together with the patient's clinical picture, can then be used to determine which of the tertiary tests tabulated in slide 7 should be used to reach a definitive diagnosis.

Slide 18: References

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Slide 19: Disclosures

Slide 20: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Primary T cell Immunodeficiencies”.