



Neonatal syndromes of polyendocrinopathy

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The neonatal immune system

Several studies have clearly indicated that fetal and newborn mammals have a limited ability to mount an immune system response, both qualitatively and quantitatively. Consequently, the neonate has increased susceptibility to bacterial or viral infection, which represents a major cause of morbidity and mortality, particularly in premature individuals. Within the first year of life, approximately 1.8×10^6 of all newborn humans die every year due to infectious diseases.

The incomplete development in the neonate of both innate and acquired immune system might explain this increased susceptibility [1].

Most studies performed on the neonatal immune system that have been conducted utilize umbilical cord blood. Although ethically correct, this approach has a number of limits.

The innate immune system

The innate immune system represents an aspecific type of defense against infectious pathogens. It is characterized by (1) physical barriers (epithelial cells and secreted products on their surface), (2) phagocytes (e.g. macrophages and neutrophils), cytotoxic cells (natural killer [NK] cells), and (3) components of the complement system.

At birth, leucocyte count is higher than in adult life, possibly due to delivery-induced stress [1]. Twelve hours after delivery, neutrophils reach levels in the range of 7000–15,000/mL [2], although an even wider range has been proposed [3].

The type of delivery appears to affect the leucocyte blood count [4], which also correlates with plasma cortisol levels. In addition, it has been shown

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that in full-term neonates (36–40 weeks of gestation), granulocyte-macrophage colony-stimulating factor (GM-CSF) storage pool in the bone marrow is significantly lower than that in adult life [5]; consequently, in response to an infectious agent, the ability of bone marrow to produce new leucocytes rapidly declines, determining neutropenia and increased susceptibility to infectious diseases [6].

G-CSF serum levels appear to be higher in neonates affected by an infectious disease, and exogenous administration of G-CSF in these patients improves the prognosis by significantly increasing the amount of neutrophils both in bone marrow and in peripheral blood [7].

In order to perform their bactericidal activity, polymorphonuclear cells (PMN) need to adhere to the vessel wall prior to migration into sites of inflammation, in response to a chemoattractant stimulus. Adhesion requires the presence and activity of members of two families of adhesion molecules: selectins (which appear to mediate the initial events of the process) and β_2 integrins.

L-selectin is a glycoprotein expressed on neutrophil cell surface that is cleaved subsequent to cellular activation [8]. In neonates born after a full-term pregnancy, L-selectin levels are lower than in adults [9]; in vitro studies indicate that anti-L-selectin monoclonal antibodies can significantly reduce the number of neutrophils able to adhere to a model of vessel wall (human umbilical vein endothelial cells) in adults but not in neonates [9].

It has been proposed that L-selectin is expressed in normal levels during fetal life and is down regulated after birth [10].

The LFA-1 integrin molecule is expressed in normal levels in neonates born after a full-term pregnancy [11], although a lack of MAC-1 upregulation has been observed in neonatal neutrophils during activation. A translocation defect and a reduced content of MAC-1 appear to be the cause of such a defect [11,12].

The defective chemotactic process observed in neonates is possibly due also to a defect in actin polymerization [13]. Neonatal PMNs show basal F-actin levels similar to adults. However, after in vitro stimulation with a synthetic chemoattractant (FMLP), F-actin levels in neonates are significantly lower than in adults [13].

As for the phagocytosis process, PMNs possess receptors both for complement system components and for immunoglobulin Fc domain. Although FC γ RII, FC γ RIII, and CRI receptors appear to be expressed in normal levels in neonatal PMNs, CR3, which is the C3b receptor, is hypoexpressed in neonatal PMNs both in the cytoplasm and in the cell membrane [14,15]. In any case, the bactericidal activity of neonatal PMNs is similar to that of adult PMNs [16].

The complement system comprises both serum and membrane-bound proteins that are involved in the effector phase of an anti-inflammatory response. Since complement system proteins cannot cross the placenta, these have to be produced by the fetus. The complement system appears to be developed between 28th and 33rd week of gestation [17]. However, in

neonates born after a full-term pregnancy, complement system enzymatic activity, measured as total hemolytic complement activity (CH50), is significantly lower than in adults. In particular, very low levels of C9 have been observed in neonates [18]. Interestingly, in animal models, administration of human C9 significantly increases bactericidal activity of rat serum [19].

NK cells are increased at birth, and represent about 20% of mononuclear cells. The number of these cells progressively decreases after the first year of life [20]. Similar to other cell components of the innate immune system, neonatal NK cells show a reduced activity in comparison to their adult counterpart [21].

Adaptive immune response

Lymphocytes have a central role in adaptive immune response, together with dendritic cells and other antigen presenting cells.

The poor immune response mounted by neonates together with the fact that neonates are easily tolerized led to the hypothesis of a qualitative difference between the neonatal and the adult T-lymphocyte [1]. However, this theory has been recently questioned, in favor of a quantitative rather than a qualitative difference.

Newborn T-cells show an increased CD38 and reduced CD45RA and CD31 expression [22] in comparison with their adult counterpart. In that the CD38 molecule is particularly expressed on thymocytes, neonatal peripheral blood cells expressing this molecule may represent immature transition elements [23]. CD31 is an adhesion molecule characteristic of naive CD4+ T-cells, which is progressively lost after repeated stimulations, both in the neonate and in the adult [23].

As for CD45, most thymocytes express CD45RO and, during the last stages of maturation, switch toward the CD45RA phenotype [24]. It has been proposed that only CD45RA+ CD4+ T-cells survive after negative selection [25]. In neonates, these cells are quantitatively less than in adults [26].

Several factors can influence the Th1/Th2 commitment of neonatal T-cells: (1) the intensity and the nature of the TCR-mediated activation signal; (2) the strength and the nature of the costimulatory signal delivered by the APC; and (3) cytokines and hormones of the environment where lymphocyte activation takes place. A major role is played by IL-12 and IL-4, which promote a Th1 and a Th2 response respectively.

A number of studies have shown [27] that IL-2, IFN- γ , IL-4, and IL-5 production is very low when cord blood CD4+ T-cells are stimulated with anti-CD3 or anti-CD2 in presence of endogenous APC. However, in vitro experiments have demonstrated that human cord blood T-cells show cytokine patterns similar to their adult counterpart when stimulated using a TCR-independent pathway or when TCR-mediated response takes place in presence of appropriate costimulatory signals. Anti-CD28 and anti-CD3 stimulation induces a major production of IL-2 by neonatal T-cells.

It is likely that the reduced TCR density on the surface, together with the low expression of adhesion molecules such as LFA-1, LFA-3, and CD2 by neonatal cells, may explain the requirement for a strong costimulatory signal. CD4+ T-cell stimulation with anti-CD3/anti-B7.1 induces a Th1-like (IFN γ , IL-2, TNF α , and TNF β), rather than a Th2-like (IL-4, IL-5), pattern of cytokine production. Cytokine production is influenced by CD28 costimulation. After repeated stimulation cycles, neonatal CD4+ T-cells appear to acquire a Th2-like phenotype. Interestingly, this phenomenon appears to affect both neonatal and adult CD4+ cells. In addition, neonatal and adult naive T-cells do not differ in terms of the ability to produce IL-4 after priming and development of Th2-like responses after repeated stimulations, and production of high levels of Th1 cytokines at priming.

In the light of these observations [28], it is likely that the Th2-polarization of the neonatal immune system is due not only to a relative immaturity of the T-cell system, but also to the involvement of other cell types such as dendritic cells and B-lymphocytes.

In general, Th1 and CTL functions are poor or absent in neonates; however, it has been shown that neonatal T-cells can generate a mature Th1 response. Neonates infected by *Bordetella Pertussis* can preferentially mount a specific Th1-like response. The expression of CD40L by activated CD4+ T-cells induces IL-12 production by CD40+ APC, with IL-12 levels depending upon the cytokine microenvironment, and by the presence and functions of adhesion molecules [29].

As far as neonatal B-lymphocytes are concerned, several studies indicate that, after specific immunization, these cells produce IgM but are not efficient in switching to the production of other subclasses. However, recent studies have indicated that in the presence of robust T-cell signaling, neonatal B-lymphocytes are able to produce IgG, IgA, and IgE, both *in vitro* and *in vivo*. The observation that neonatal B-cells can undergo class switching in the presence of adult T-help suggests that inadequate help from resident neonatal T-cells may be responsible for the neonatal inefficient Ig class switch.

Autoimmune endocrine syndromes of the neonate

An increasing number of studies have shown that a variety of immune-mediated disorders, including autoimmune syndromes, can take place in the early post-natal life, a period during which the immune system acquires key functions and undergoes a complex maturation and education process. As described in the previous paragraphs, the neonatal immune system, under certain circumstances, can mount an efficient immune response; therefore it is not surprising that, in the appropriate genetic and environmental scenario, organ specific autoimmunity can occur very early in life.

We will describe autoimmune neonatal syndromes involving endocrine glands as target.

IPEX/XLAAD/XPID

IPEX (immunodysregulation, polyendocrinopathy, and enteropathy, X-linked) or XLAAD (X-linked autoimmunity-allergic dysregulation syndrome) or XPID (X-linked polyendocrinopathy immune dysfunction diarrhea) represent the human equivalent of the scurfy mouse, a murine model [30] characterized by hyperproliferation of CD4⁺ T-lymphocytes, extensive multiorgan infiltration, increased cytokine production, and premature death between day 16 and 25 of life.

In this model, the recessive X-linked disease is due to a mutation of the *Foxp3* gene, encoding for a transcription factor belonging to the forkhead/winged-helix family.

Recently, Widin et al. [31], Bennett et al. [32] and Chatila et al. [33] have sequenced the human ortholog (FOXP3, mapping to chromosome Xp11.23-Xq13.3) of the gene mutated in the scurfy mouse (*Foxp3*), in order to determine whether a mutation in this gene might be associated with a clinical syndrome described in the 1980s [34], and characterized by untreatable diarrhea, eczema, insulin-dependent diabetes mellitus thrombocytopenia, and thyroid autoimmunity. These studies, performed on a number of affected families, have uncovered mutations of the genes that were responsible for the defective function of the forkhead/winged-helix domain of the scurfin protein. The observation of an increasing number of mutations suggests a genetic heterogeneity of this syndrome, which may also appear later in life.

In humans, the syndrome usually appears early in life with autoimmune phenomena, signs and symptoms of allergies (eczema, elevated IgE levels, peripheral eosinophilia), secretory diarrhea, and growth retardation. Increased susceptibility to staphylococcal infections has also been described, and the syndrome is frequently fatal due to untreatable diarrhea or to superimposed infections.

It has been observed recently [35] that the prognosis can be improved by syngeneic bone marrow transplantation. Baud et al. have reported [35] a case of a patient carrying a F371C gene mutation that at 2 weeks of age developed ichthyosis, at 4 weeks severe secretory diarrhea and hemolytic anemia and, at 3 months of age, type-1 diabetes mellitus. The patient was also suffering from eczematous skin lesions, which only partially responded to cortisone therapy. Following bone marrow transplantation from his HLA-matched sister, the patient recovered from the symptoms and has been disease-free for 29 months after transplantation. An unexplained episode of hemophagocytic syndrome caused his death.

As to neonatal autoimmune diabetes, we recently described [36] a congenital organ-specific autoimmune disorder, resembling XLAAD syndrome, involving different organs and selectively affecting β -cells in the pancreas. Clinically, the most striking feature of this neonate was the finding of absolute insulin deficiency after twelve hours of life, suggesting a rare form of congenital type-1 diabetes. The autoimmune origin of this form

of insulin-dependent diabetes was first indicated by the presence of high titres of insulin autoantibodies and anti-GAD65 in the infant but not in the mother, ruling out the possibility of transplacental transmission of these antibodies. Morphological and histochemical investigations performed on several organs showed a dramatic scenario: massive mononuclear cell infiltration in the pancreas with signs of extensive insulitis, heart and lung reinforcing the autoimmune origin suspected in this syndrome. The characterization of such infiltrates revealed a high proportion of T-lymphocytes (CD3+), the majority of which, interestingly, were of memory phenotype (CD45RO+).

In the pancreas, we were able to identify pancreatic islet structures both by H&E staining and by the presence of chromogranin-A, ruling out the possibility of a congenital absence of islets of Langerhans. No insulin-producing cells were found (Fig. 1A and 1B), but glucagon positive cells were present

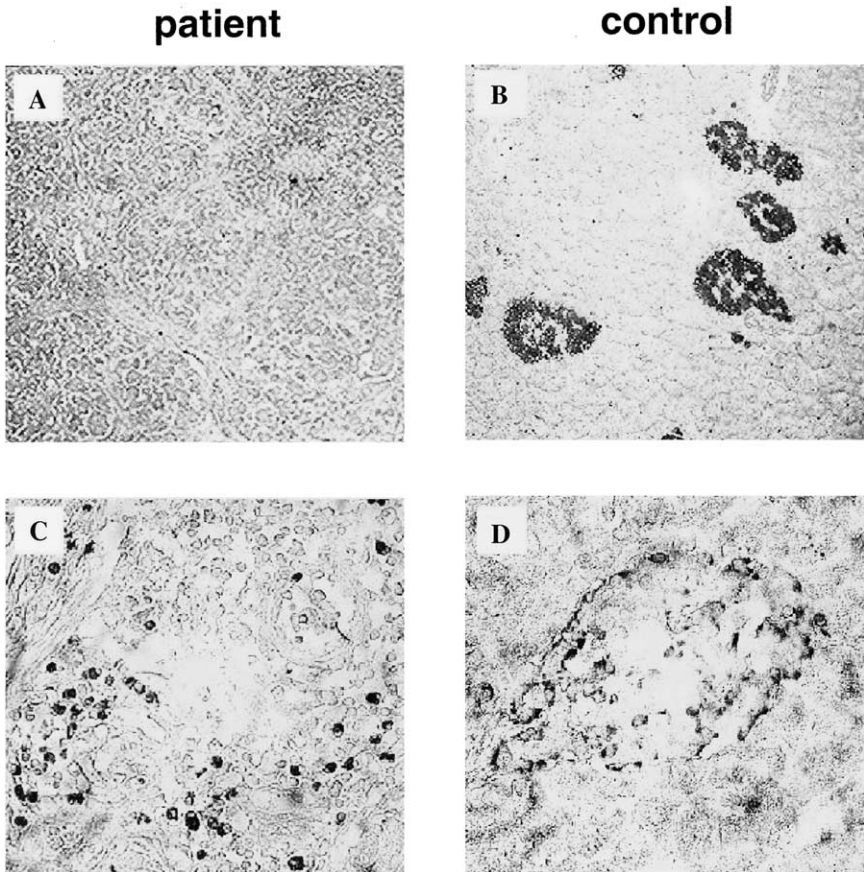


Fig. 1. Insulin deficiency in the pancreas of the infant. Expression of insulin and glucagon in the patient pancreas (A and C) compared with a control pancreas (B and D) (indirect immunoperoxidase × 40).

(Fig. 1C and 1D), indicating pancreatic β -cell specific autoimmune destruction. No sign of enteroviruses, which have been reported to be involved in myocarditis and eventually in autoimmune diabetes, was found in any of the paraffin embedded tissue analyzed. Histological examination of the gut revealed, beside a severe necrotizing enterocolitis affecting approximately two-thirds of the small bowel, otherwise normal intestinal mucosa.

HLA typing showed in the neonate the expression of DQB 0501, 0201, and of DR1/DR3; the mother was DQB 0501, 0301, and DR1/DR4, and the father was DQB 0301, 0201, and DR4/DR3. The neonate had at least one diabetes-susceptible HLA allele (DQB1 0201, DR3) inherited from the father, which probably influenced the β -cell specificity of pancreatic autoimmunity. No maternal chimerism was observed in the infant blood by HLA typing, confirming the absence of a materno-fetal GVDH.

Neonatal hyperthyroidism

The main cause of neonatal hyperthyroidism is Graves' disease [37]. It has been estimated that prevalence of neonatal autoimmune hyperthyroidism is 6–25/10,000.

Patients affected by this disease are flushed, hyperkinetic, and diaphoretic, and suffer from diarrhea, vomiting, and poor weight gain. Goiter is frequently observed, and ophthalmopathy and periorbital edema could be present.

Thyrotoxicosis may manifest in neonates with congestive heart failure and cardiac arrhythmias. Neonatal hyperthyroidism usually develops in intrauterine life during a pregnancy occurring in a hyperthyroid mother, and is caused by transplacental passage of thyroid stimulating autoantibodies. The fetus, as well as the neonate, is at increased risk of thyrotoxicosis when autoantibody levels show a 5-fold increase compared to normal values. However, at 48 weeks of age, all hyperthyroid neonates, in which the disorder is caused by transplacental passage of thyroid-stimulating autoantibodies, should be in a euthyroid state.

However, it has been shown [38] that some individuals with neonatal hyperthyroidism have endogenous production of thyroid-stimulating immunoglobulins. In these cases, hyperthyroidism can last for a long time, and disease remission can be usually achieved only after thyroidectomy.

Other causes of neonatal hyperthyroidism are due to inactivating mutations of the TRH gene or of the α -subunit of the stimulatory G-protein.

From a diagnostic point of view, TSH levels will be suppressed in the presence of increased FT3 and FT4 values; thyroid-stimulating immunoglobulins will be positive in the neonate and/or in the mother. Lack of these autoantibodies may suggest the existence of inactivating mutations of the TRH gene or of the α -subunit of the stimulatory G protein.

As far as the treatment is concerned, propylthiouracil can be administered at daily doses of 5–10 mg/Kg; however, neonatal hyperthyroidism

caused by an autoimmune response occurring in the neonate rather than by the transplacental passage of maternal autoantibodies, may require total thyroidectomy.

Di George syndrome

Di George syndrome is characterized by thymic hypoplasia, parathyroid hypoplasia, and outflow tract defects of the heart. Most cases are due to chromosome deletions within the 22q11.2 region [39]. Several genes can be lost, including the putative transcription factor *TUPLE1*. This gene contains 4 WD40 domains, and shows evidence of expression at the critical period of development in the outflow tract of the heart and of neural crest derivatives in the upper thorax. Another gene of interest in the 22q11.2 chromosomal region is *Tbx1*, which encodes for a transcription factor of the T-box family. Mice with mutations of this gene show cardiac, parathyroid, and thymic abnormalities that closely resemble those of the human Di George syndrome. The observation [40] of two patients with this syndrome presenting idiopathic thrombocytopenic purpura raised the hypothesis that defective thymic function occurring in these patients may predispose them to autoimmune diseases.

ALPS

Autoimmune lymphoproliferative syndrome (ALPS) was first described, at the end of 1960s [41], as an autoimmune syndrome characterized by hemolytic anemia, thrombocytopenia, lymphadenomegaly, and splenomegaly. This syndrome is due to mutations of the Fas receptor or of its ligand FasL, which are responsible of ALPS1-A and ALPS1-B respectively. Among Fas gene mutations described [42,43], those located in the intracellular region of the molecule are usually inherited as an autosomal dominant trait, while those located in the extracellular portion of the molecule, although determining defective Fas expression, do not have a dominant-negative effect. In families with an extracellular domain mutation, only a single individual was affected. A variant of this syndrome, called ALPS-II, is caused by mutations of the caspase-10 gene.

The Fas system has a major role in the maintenance of tissue homeostasis. The binding of FasL to Fas leads to apoptotic death by activating a cascade of events involving the sequential activation of proteases called caspases.

The above-mentioned mutations impair apoptosis in a number of cell systems that are responsible for the lymphoproliferative phenomena, representing the characteristic feature of these groups of syndromes.

As for autoimmune phenomena occurring in this syndrome, the most common are hemolytic anemia and thrombocytopenia; however, thyroid autoimmunity and type-1 diabetes have also been reported. Recently, De Franco et al. [44] reported an atypical form of ALPS associated with

type-1 diabetes, in which lymphocytes were resistant to Fas-induced apoptosis despite lack of mutations of the Fas gene and normal expression of the Fas molecule in peripheral blood mononuclear cells.

Kabuki syndrome

Kabuki syndrome is a genetically transmitted disease described in 1988 [45] in a group of 62 patients (mainly Japanese) characterized by: (1) distinct facial anomalies with prominent ears, depressed nasal tip, eversion of the lower lateral eyelid, and arched eyebrows; (2) skeletal abnormalities and severe to moderate mental retardation occurring in 92% of patients; (3) impaired postnatal growth in 83% of cases; and (4) dermatoglyphic abnormalities in 93% of patients. Other clinical features can include hydronephrosis, and hepatic anomalies. Autoimmune disorders such as Hashimoto's thyroiditis and vitiligo can be present.

In some cases, the type of inheritance appears to be autosomal dominant.

The disease incidence is 1:32,000 in Japan; sporadic cases have been described in Caucasoids as well as in Arabs [46,47].

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