



## Immunological peculiarities of extremely preterm infants: a challenge for the prevention of nosocomial sepsis

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### Abstract

**Objective:** To review the main aspects of fetal immune development focusing on the host defenses of extremely preterm infants against bacterial pathogens, and describing the possibilities of immunotherapeutic intervention for the prevention of neonatal nosocomial sepsis.

**Sources of data:** Electronic search of MEDLINE database for articles published in the last 15 years. Those with relevant information regarding the target issue were selected.

**Summary of the findings:** Immunity of extremely preterm infants is deficient due to skin fragility, insufficient complement system components, decreased bone marrow neutrophil storage pool, and lower chemotaxis, adherence, deformability, and neutrophil enzyme activities. Further limitations are found at NK cell-mediated cytotoxicity, T cell proliferation and cytokine production, B and T cell cooperation, and antibody synthesis by B lymphocytes. No definitive benefits of interventions for enhancing the immune function, such as the use of intravenous immunoglobulin or myeloid colony-stimulating factors, have been demonstrated.

**Conclusion:** As a consequence of the immaturity of several immune components, extremely preterm infants are highly susceptible to nosocomial infections. The very limited possibilities for intervention in this system require the control of extrinsic factors for the prevention of nosocomial sepsis in these infants.

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### Introduction

For decades, improvements in intensive care have allowed for the longer-term survival of extremely preterm newborn infants (those weighing less than 1,000 g at birth). These improvements have been made not only in industrialized countries, but also in Brazil. Twenty years ago, 50% of infants weighing less than 1,500 g at birth, treated at Hospital das Clínicas of the School of Medicine of Ribeirão Preto (HCFMRP-USP) would not survive, compared to 19% at the present time. This improved survival rate has been associated with a higher incidence of nosocomial infection in neonatal intensive care units. The risk of sepsis is inversely proportional to gestational age. The prevalence

of sepsis and meningitis has been estimated in different populations of newborns, ranging from 1 to 5/1,000 live births. However, for preterm infants, this prevalence is expected to be as much as 1/230 newborns, and sepsis is quite common in this group of patients.<sup>1</sup>

As examples of the high frequency of nosocomial infection in extremely preterm newborn infants, recent data from U.S. neonatal surveys<sup>2</sup> indicate an overall sepsis incidence of 21%, with a significantly higher frequency, the younger the gestational age (Table 1). In Brazil, according to an observational study carried out in 2001 in seven neonatal intensive care units, antibiotic therapy had to be administered for over five days in 290 of 514 (56.6%) newborns with gestational age less than 34 weeks, and in 81 of them (27.9%) the microbiological blood culture yielded positive results.<sup>3</sup> Epidemiological surveillance data on nosocomial infections in the neonatal intensive care unit of HCFMRP-USP, in the year 2001, indicated an infection incidence rate of 57.3 and 43.9 per 100 infants at risk and those weighing  $\leq$  1,000 g at birth and between 1,001 and 1,500 g, respectively.<sup>4</sup> In this unit, the incidence densities of nosocomial infection

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**Table 1** - Percentage of children with nosocomial sepsis, with at least one positive blood culture, among 6,215 newborns < 1,500 g, according to weight and gestational age

Weight at term (g)	Frequency (%) infection	Gestational age (weeks)	Frequency (%) infection
401-750	43	< 25	46
751-1,000	28	25-28	29
1,001-1,250	15	29-32	10
1,251-1,500	7	> 32	2

Stoll et al.<sup>2</sup>

observed in this period were 34.6 and 42.7 per 1,000 patients-day in preterm infants weighing  $\leq$  1,000 g and between 1,001 and 1,500 g, respectively.<sup>4</sup> Also, among extremely preterm infants, higher nosocomial infection incidence rates were observed at another Brazilian NICU.<sup>5</sup> Usually, mortality from neonatal sepsis ranges from 15 to 60%, depending on gestational age, on the pathogen detected, on the age at which the disease occurs in the neonatal period and on the quality of intensive care.<sup>1</sup> The mortality rate for septic newborns weighing less than 1,500 g was 18%, significantly higher than that for infants who did not have sepsis (7%) in the U.S. neonatal survey.<sup>2</sup> In the NICU of HCFMRP-USP, 17.6% of 324 newborns under surveillance in the year 2001 died; death was significantly more frequent among infants diagnosed with nosocomial infection than among those without this diagnosis (21.6 *versus* 12.8%). Infection was the cause of death in most infants (75.7%) who had one or more infectious episodes, mainly those with birthweight < 1,000 g.<sup>4</sup>

This dataset shows that it is important to understand the factors associated with nosocomial sepsis in extremely preterm newborns. Several factors predispose these infants to nosocomial sepsis:

1. Extrinsic factors often expose preterm newborns to infection during their hospital stay. Among these factors are the length of hospital stay, use of invasive procedures (arterial and venous catheters, parenteral nutrition, tracheal cannulas, gastric or gastroduodenal tube, ventriculoperitoneal shunts, chest drains, etc.), characteristics of the exposure to the hospital environment and to the hospital staff, i.e., the nurse/patient ratio, physical area, personnel training, hygiene techniques, nosocomial infection control techniques and the pattern of antimicrobial use at the NICU. Overcrowding, insufficient number of caregivers and the antibiotic selective pressure increase the chances of acquisition of potentially virulent pathogens.<sup>6</sup>
2. Intrinsic factors are concerned with the immature development of the immune system and of protective functions of the skin, mucous membranes and gastrointestinal tract. Birthweight is probably the

substitute marker for these intrinsic factors. However, disease severity varies among infants who have the same birthweight and is an important risk factor for morbidity and mortality.

In general, some bacterial infections are not well limited by newborns, and therefore there is a tendency towards systemic dissemination. Some functions of the immune system are immature, whereas other aspects are functional at birth, even in extremely preterm newborn infants. In the present article, we will review the major immunological peculiarities of extremely preterm newborns, in order to assess the rationale behind their susceptibility to infection.

### General aspects of the immune system

The main function of the immune system is to protect the host against microorganisms.<sup>7</sup>

The first line of defense against invaders is the physical barrier made up of the keratinized skin, mucous membranes that line the respiratory and gastrointestinal tracts and chemical barriers containing a series of enzymes and other substances that have a direct antimicrobial action or inhibit microbial adherence to body surfaces.

Any invader that crosses this first line of defense will be attacked by the components of the innate immune system and, afterwards, by the specific immune system.

Innate immunity involves humoral elements such as complement system proteins, acute phase proteins, cytokines and cellular elements such as monocytes, macrophages, granulocytes, dendritic cells and natural killer (NK) lymphocytes. This system has a limited capacity to distinguish between microorganisms and often has a similar response to different microorganisms.

The components of specific immunity are the lymphocytes and their products (e.g.: antibodies). In opposition to innate immunity, this system responds specifically to each microorganism, has a memory and is able to respond more vigorously to repeated exposures to the same antigen.

Innate and specific immunities work conjointly. Innate immunity protects against microorganisms and also plays a key role in the induction of specific response, and this response enhances the action of protective mechanisms of innate immunity.<sup>8</sup>

With regard to newborns, another immunological component that acts while the maturation of their system is underway is the immunity passively acquired from the mother, by means of IgG antibodies and human milk.

### Development of the immune system

The immune system is made up of cells derived from the precursor cells of the hematopoietic system whose major source is the yolk sac up to the third week of life, followed by fetal liver at 8 weeks and finally by the bone marrow after the fifth month of gestation. During intrauterine life, these cells are submitted to the effect of specialized

microenvironments, such as bone marrow and thymus, responding to signs of stimulation, proliferating and differentiating in order to form the innate and specific immune system. This is a complex and detailed process. Table 2 shows some moments of differentiation of this system during intrauterine life. The functions of the different components in preterm newborns will be discussed next.

**Mechanical barriers and immunity of mucous membranes**

The skin of the preterm newborn, especially of the extremely preterm infant, is immature and inefficient as epidermal barrier. In normal fetal development, the stratum corneum, responsible for the epidermal barrier function, only becomes mature around the 32nd or 34th week of gestation.<sup>9</sup> Even though maturation accelerates after birth up to the second postnatal week, the immature skin of extremely low birthweight infants is more susceptible to ruptures, which facilitates the penetration of germs.

There is little information about the maturation of the fetal immune system regarding the mucosal compartment. The main component of this compartment is the secretory immunoglobulin A (SIgA). Seidel et al.<sup>10</sup> found similar SIgA levels in the saliva of full-term and preterm infants (mean gestational age of 30 weeks) in the first nine months of life. Nevertheless, other authors reported lower SIgA levels in preterm infants at the age of 3 to 8 postnatal months.<sup>11</sup>

Another important barrier to infection is that of the gastrointestinal tract, which is impaired in preterm infants, since the protective gastric acidity is adversely modified by the virtually continuous feeding of newborns, which increases pH, or by the occasional use of H2 blockers.<sup>12</sup> Assessment of the gastrointestinal immunologic component shows that

only small amounts of the secretory component are detected up to the 29th week of gestation; also, the immunocytes that produce IgA, IgM or IgG are scarce up to the first postnatal week. However, after the second postnatal week of infants born between the 24th and 32nd gestational week, there is intense epithelial expression of histocompatibility antigens and development of SIgA, suggesting that this defense system is modulated in response to environmental factors after birth.<sup>13</sup>

**Innate immunity**

**Complement system**

This system consists of approximately 20 proteins produced mainly by the liver and found abundantly in blood and tissues, requiring sequential activation. There are three forms of activation of the complement system: alternative, lecithin-dependent, and classic. The first two are activated in a nonspecific way by contact with certain components on the surface of microorganisms, regardless of the presence of specific antibodies. Consequently, a series of substances is formed (C3a, C3b, C5a, among others) that release inflammatory mediators, stimulate chemotaxis and phagocytosis and, when the activation is complete, cause microbial lysis through the components of the membrane attack complex (C5b-C9).<sup>7</sup> The classic pathway is initiated by the binding of circulating antibodies to the first protein (C1) of the complement system.<sup>8</sup>

The elements of the complement system are detected in the fetus early on during gestation, but the levels of these proteins remain low up to the last trimester. There is no transplacental transfer of complement system elements, but the fetus and the newborn can synthesize them and this ability increases with gestational age.<sup>14</sup> At

**Table 2 -** Stages of fetal immune system development

<b>Fetus age (weeks)</b>	<b>Innate immunity</b>	<b>Humoral immunity</b>	<b>Celular immunity</b>	<b>Passive immunity</b>
5-6	Macrophages in the liver and blood		T-cell precursor in the liver	
9-10	Start of the complement synthesis	B precursors in the liver	T-cell precursors in the thymus	
12-14	Macrophages in lymphonodes and APC MHC class II	Pre-B cells with IgD, IgG and IgA	T-cells CD4+ and CD8+ in the liver and spleen	Start of mother's IgG transfer
16-17	Mature macrophages in the liver and circulating neutrophils	Large number of B-cells in the spleen, blood and bone marrow	T-cells in the blood and lymphoid tissues / Rearrangement of receptors	
20-30		B-cells secrete antibodies	Gradual increase of T-lymphocytes secreting lymphokines	Gradual increase of IgG transportation

APC = antigen-presenting cells; MHC = major histocompatibility antigens.

the end of gestation, there is an increase in C3 of up to 60-80% of the value observed in adults, but the final components of the complement system may reach only 10% of maternal levels. Despite this quantitative deficiency, the functional capacity in full-term newborns, determined by the total hemolytic complement activity (CH50), is similar to that observed in adults. The components of the alternative pathway in newborns reach 35 to 70% of adult levels. Preterm newborns have lower complement and lytic activities levels at birth, and the levels of C3, C4 and CH50 increase with gestational age.<sup>15</sup> Low levels of early components in preterm newborns cause deficiency of activation products that are crucial to chemotaxis and opsonization.

### *Phagocytes*

Neutrophils, monocytes and macrophages are able to phagocytose microorganisms and destroy them intracellularly by the action of several toxic substances, including superoxide anions, hydroxyl radicals, nitric oxide, cationic protein, hypochlorous acid and lysozyme. Neutrophils and the mononuclear phagocyte system cells originate in the bone marrow from granulocyte and monocyte-macrophage cells, the colony-forming unit granulocyte-monocyte (CFU-GM) pool. Glycoprotein hormones, known as colony stimulating factors (G-CSF/ GM-CSF), induce the proliferation, maturation and differentiation into neutrophils and monocytes.<sup>16</sup>

Macrophages and dendritic cells are the main soluble antigen-presenting cells that induce the proliferation of T lymphocytes by the secretion of cytokines that provide signs for the activation. To do that, it is necessary to have the expression of major histocompatibility (MHC) antigens of classes I and II on the cell surface. Around the 12th week of gestation, the expression of these molecules is evident in several fetal tissues, including macrophages and dendritic cells in a similar way to that of adults.<sup>17</sup> However, some data indicate deficiencies in the processing and presentation of antigens, and failure in the regulation of the expression of MHC II on the cell surface, thus minimizing this function in newborns.<sup>18</sup> With regard to neonatal monocytes, deficiencies such as low production of cytokines have also been observed.<sup>19</sup>

Neutrophils (polymorphonuclear leukocytes) are able to respond and migrate quickly and in large numbers to the lesion or infection site. Since these cells are essential components of innate immunity, they have been extensively studied in newborns regarding body components and functions of chemotaxis, deformability, adherence, phagocytosis and bacterial lysis.

The bone marrow neutrophil storage pool in newborns is smaller than that of adults and may become totally depleted in the presence of sepsis. In infants born with less than 32 weeks of gestation this storage is equivalent to nearly 20% of the pool of full-term newborns and adults.<sup>20</sup> In these extremely preterm newborns, the occurrence of neutropenia during the course of an infection is often due to the depletion of bone marrow reserves.<sup>21</sup>

Neutrophils remain inactive in the bloodstream and circulate at a high speed.<sup>8</sup> Neutrophil activation occurs in the tissues, at sites of infection, but before they get there they need to come into contact with the endothelium, adhere, stop, deform and cross endothelial cells. The transient contact of neutrophils with the endothelium, recognized in dynamic videomicroscopic images such as "cell rolling," depends on the association and dissociation of receptors and their ligands in endothelial cells and at the apices of villous projections of neutrophils. The main molecules involved in these interactions are the placental selectin (P-selectin), endothelial selectin (E-selectin) and leukocyte selectin (L-selectin).<sup>16</sup> P-selectin, expressed in platelets and endothelial cells, is subtly expressed by the fetal endothelium before the 27th week of gestation and its expression is basically normal in full-term infants and low in preterm newborns.<sup>22</sup> L-selectin is basically expressed by leukocytes, with low levels in the cord blood of newborns, especially in preterm newborns.<sup>23,24</sup> In the first month of life, the expression of L-selectin is low in the cells of newborn infants comparatively to the cells of adults.<sup>25</sup> The firm adhesion of neutrophils to the endothelium is mediated by beta-2-integrins. The most important adhesin in this group is the complement receptor 3 (CR3 or CD11b/CD18). CR3 is expressed at low concentrations on the surface of inactive neutrophils. CR3 recognizes the members of the superfamily of intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 to which they bind, resulting in the firm adhesion of the neutrophil to the endothelium. Inactive neutrophils in newborns have a CR3 expression similar to the neutrophil expression in adults.<sup>25</sup> The stimulation and activation by inflammation increase *in vivo* and *in vitro* CR3 complexes, but this increase is significantly lower for neonatal neutrophils.<sup>25,26</sup> The total CR3 concentration in neonatal neutrophils increases with gestational age up to 60% of the value observed in adults.<sup>26</sup>

After adhering to the vascular endothelium, neutrophils have to deform in order to cross the junctions between endothelial cells. To some extent, deformability depends on the formation of actin filaments from the polymerization of monomeric G-actin. Neutrophils in newborns form fewer actin filaments after stimulation with chemotactic factors than neutrophils in adults.<sup>27</sup> The neutrophil membrane is another determinant factor for deformability. The increased fluidity of neutrophil membrane in newborns results in reduced deformability.<sup>28</sup>

Outside the blood vessels, neutrophils move towards the site of infection, guided by chemotactic molecules such as the C5a fragment of the complement system, interleukin-8, leukotriene B4, platelet activating factor, and fragments of bacterial proteins known as formyl-methionine peptides (f-met).<sup>8</sup>

At the site of microbial invasion, a contact is established between the pathogen and phagocyte through opsonin binding (immunoglobulin G (IgG) and C3b/iC3b) to the pathogen with specific receptors on the cell surface. The interaction between opsonins and receptors along with the activation of contractile elements produces pseudopods that engulf the pathogen forming a phagocytic vacuole that

is internalized and suffers the sequential action of antimicrobial proteins and hydrolytic enzymes released by neutrophil granules.<sup>16</sup> The bactericidal permeability increasing protein (BPI) that neutralizes the lipopolysaccharide endotoxin (LPS) and is cytotoxic to gram-negative bacteria has a concentration three to four times lower in full-term newborns compared to adults.<sup>29</sup> The amount of BPI found in the neutrophils of preterm newborns is even lower than that of full-term newborns.<sup>30</sup>

During phagocytosis, there is a sudden increase in the cellular metabolism of oxygen (respiratory burst) with the production of oxygen metabolites including superoxide anion [O<sub>2</sub><sup>-</sup>], hydrogen peroxide [H<sub>2</sub>O<sub>2</sub>] and the hydroxyl radical [HO], which have bactericidal activity. The interaction of oxygen metabolites with excitable substrates in the cell also leads to a chemiluminescence burst. It is controversial whether the increase in respiratory activity of neutrophils in newborns is identical to that of adults and whether that of preterm infants is lower than the one observed in full-term infants.<sup>31,32</sup> Recently, Björkqvist et al.<sup>33</sup> have shown that neutrophils in preterm infants have a lower capacity to increase their respiratory activity after stimulation with coagulase-negative staphylococci than do neutrophils in full-term infants.

#### *NK cells*

These cells are large and granular lymphocytes that often express the IgG receptor (CD16) and the CD56 marker. Their ability to produce cytotoxicity enables them to lyse infected cells or antibody-sensitized cells in a direct fashion. These cells act mainly against tumor cells and virus-infected cells. However, they are also able to lyse bacteria, parasites, fungi and some normal cells in the absence of previous sensitization. Comparatively to adults, newborn infants have similar or higher amounts of NK cells in peripheral blood. Nevertheless, there are phenotypic and functional differences, for instance, the cytotoxic activity is lower in newborns.<sup>34</sup> Few studies have been carried out with preterm infants and have shown that these infants have lower NK activity than full-term newborns, and also a smaller amount of cells.<sup>35</sup> In these infants, there is an increase in the amount of NK cells and in the lytic capacity of these cells after birth, which may result in improved cytokine production<sup>35</sup> in response to the stimulation from the extrauterine environment.

### **Specific immunity**

#### *T lymphocytes*

They are specialized cells of the immune system that, after being stimulated by antigen-presenting cells and being activated, respond to new antigens by producing or expressing cytokines in their cell membrane that amplify or regulate the several aspects of immune response.<sup>7</sup> In addition to effector functions, these cytokines take part in key effects for the proliferation of NK cells, monocytes, B lymphocytes and for the proliferation of T lymphocytes as well. T lymphocytes are subdivided into various subtypes

according to their surface markers and to their cytokine production.<sup>8</sup> The two largest subpopulations of T lymphocytes that are important to specific immunity are the CD4+ or T helper lymphocytes and the CD8+ or cytotoxic lymphocytes. The function of CD4+ cells is to activate macrophages and encourage B lymphocytes to produce antibodies. The population of CD8+ cells plus that of NK cells mediates most cytotoxic activities against virus-infected cells or tumor cells. At the initial stage of an infection, the infected cells are nonspecifically lysed by NK cells, which controls the infection, but eradication occurs through the clonal expansion of antigen-specific cytolytic cells (CD8+).<sup>8</sup>

Neonatal T lymphocytes exhibit deficiencies such as low proliferative response, lower interleukin-2 (IL-2) production, decrease in the cytolytic activity, and abnormal production of cytokines.<sup>18</sup> Thus, the responses of the fetus and newborn infant to specific T-dependent antigens, including CD8+-mediated cytotoxicity<sup>36</sup> and the production of CD4+-dependent antibodies<sup>37</sup> is reduced or delayed, in comparison to other individuals.

With regard to cell-mediated immunity in extremely preterm infants, some peculiarities distinguish them from adults and full-term infants. The major differences are the decrease in the *in vitro* proliferation after phytohemagglutinin stimulus and the smaller number of CD3 and CD8 cells.<sup>38</sup> However, comparatively to adults and full-term infants, the response to some microbial pathogens (*H. influenzae*, *S. epidermidis*) is enhanced on the first day of life, but this response decreases two weeks after birth.<sup>39</sup>

#### *B lymphocytes*

The major function of the population of B cells is the production of several spectra of immunoglobulin molecules that constitute the humoral component of the specific immune response. From precursor cells, plasma cells are the most differentiated ones for the production of antibodies. In adults, these cells are able to synthesize thousands of molecules per second. These cells are activated by the interaction with T lymphocyte and the antigen. In response to the antigen challenge, the production of IgM is the primary response.<sup>7</sup>

The infected fetus and newborn infant are able to produce IgM antibodies in response to bacterial antigens, but at lower levels than that of adults. However, the synthesis of IgG and IgA is limited.<sup>14</sup> On top of that, these antibodies do not respond to certain antigens, especially bacterial polysaccharides and have limited capacity to develop memory. This limited production of antibodies may be due to the development of B cells and to the absence of signs of stimulation.<sup>14</sup>

### **Passively acquired immunity**

The transplacental transfer of maternal immunoglobulin to the fetus is a specific adaptation that, to some extent, minimizes these deficiencies in the production of antibodies. The transfer of IgG is limited to IgG isotopes. The IgG Fc fraction has been regarded as the main fraction implicated

in the transplacental transfer of this immunoglobulin. The presence of receptors for the Fc region of IgG has been observed in human placenta in the first 12 weeks of gestation. The syncytiotrophoblast and Hofbauer cells are the main cells that contain Fc receptors.<sup>40</sup> All of the four IgG subclasses (IgG1, IgG2, IgG3 and IgG4) cross the placenta. The fetus receives the antibodies against the antigens to which the mother was exposed due to colonization, infection or vaccination. If the mother has small concentrations of antibodies or if the protective antibodies are not of the IgG isotype, as occurs with IgM antibodies against gram-negative bacteria, such as *E. coli* and *Salmonella sp*, the fetus will not receive them.<sup>14</sup>

Studies on the pattern of transfer of different types of specific IgG antibodies show that there are peculiarities in the transfer of these antibodies. The placental transfer is more efficient for IgG1 and IgG3 and less efficient for IgG2.<sup>41</sup> Therefore, the transfer of antibodies against viral proteins of the IgG1 subclass (poliovirus, measles, rubella, mumps) and antitoxins (tetanus, diphtheria, erythrogenic) occurs more easily. However, antibodies against encapsulated bacteria (*Haemophilus influenzae*, *Bordetella pertussis*, *Neisseria meningitidis*, *Streptococcus pneumoniae*), in which IgG2 prevails, are transferred less efficiently, i.e., only 50 to 60% of those detected in the mother.<sup>42</sup>

The active transport of immunoglobulin G via the placenta begins early on and increases proportionally, and in this case, gestational age influences the total IgG levels in umbilical cord blood, with a linear correlation between the concentrations of fetal IgG and gestational age. Around the 32nd week of gestation, the detectable levels in the newborn are of approximately 400 mg/ml, but levels greater than 1,000 mg/dl can be achieved in full-term infants, being sometimes even higher than maternal levels.<sup>14</sup> Preterm infants, especially extremely preterm ones, may not receive protective antibodies, as most of these antibodies are transferred to the fetus after the 34th week of gestation.<sup>43</sup> Thus, as also occurs with full-term infants, this deficiency is even more pronounced for IgG2 antibodies.

Table 3 summarizes the main characteristics of the premature newborns immune system components previously mentioned.

Human milk and the colonization by the intestinal microbiota obtained from the mother are other essential adaptations for the passive protection of newborn infants. Human milk contains several protective elements. In addition to protecting infants in a passive way, human milk stimulates the immune system of newborns by way of anti-idiotypic antibodies and through the uptake of lymphocytes, cytokines and other elements.<sup>44</sup>

Among other protective factors, SIgA, the isotype predominantly found in human milk, provides protection against all the microorganisms that the mother may or may not have in her digestive tract, preventing them from adhering to the mucous membranes. Lactoferrin, the main protein component of mature milk, is relatively resistant to enzymatic digestion and has antimicrobial,

immunostimulatory and antiphlogistic effects, by decreasing the synthesis of proinflammatory cytokines such as IL-6, IL-8 and TNF.<sup>45</sup> The oligosaccharide fraction of milk contains analogues of several receptors for microorganisms in the mucous epithelium. This way, the presence of human milk during neonatal colonization and the subsequent expansion of the intestinal microbiota is an essential factor not only for the prevention of infections, but also for the induction of the immune system maturation.<sup>44</sup> Extremely preterm infants, due to different factors, often are not allowed to be fed milk from their own mothers or from a milk bank, which also contains most of the immune properties.<sup>46</sup> Consequently, intestinal colonization occurs through an unbalanced microbiota made up of bacteria found in intensive care units, many times virulent bacteria submitted to the antimicrobial selection pressure, which favors the invasion of these microorganisms.

### **Proposals for improvement of the immunity of extremely preterm infants and prevention of nosocomial infection**

As previously described, preterm infants have several factors that favor bacterial invasion and the occurrence of neonatal sepsis. The immature mechanical barriers, the limited functions of neutrophils, the low plasma concentration of specific antibodies, the low activity of complement system proteins and the poor cooperation between T and B lymphocytes predispose these infants to bacterial invasion.

Knowledge about several characteristics relative to the development of the fetal immune system and about the deficiencies of defense mechanisms for the protection against neonatal pathogens opened the path for potential interventions aimed at enhancing defenses and preventing or treating nosocomial infection.

We will review the main proposals regarding immunomodulation for the prevention of infection in extremely preterm infants.

#### **Topical skin emollients**

Prevention of skin barrier breakdown and of bacterial penetration through the skin using topical emollients has been proposed. However, although their use in the first 15 days improves the condition of the skin and prevents loss of water through the skin, there is no evidence of protection against bacterial invasion. Conversely, data obtained from a study including 1,191 newborns weighing between 501 and 1,000 g at birth and gestational age  $\leq 32$  weeks assigned to receive generalized application of emollients twice a day or selective application on the site of the skin lesion, instead of having a protective effect against infection, revealed a higher risk of bacterial nosocomial sepsis.<sup>47</sup> Similarly, a recent meta-analysis of the available studies showed that the prophylactic application of topical emollients in extremely preterm infants increased the risk of any nosocomial infection and, mainly, of infections caused by coagulase-negative staphylococci.<sup>48</sup>

**Table 3** - Actions of the premature newborns immune system components

Component	Action in premature newborns	Reference(s)
Mechanical barriers	Maturation of the stratum corneum around the 32nd or 34th week of gestation	9
Intestinal mucosa	Small amounts of MHC receptors, secretory component and IgA, IgM and IgG immunocytes Significant increase after 2 weeks after birth	13
Macrophages and tissues dendritic cells	Expression of receptors and adult-like function since the 12th week of gestation	17
Proteins of the complement system	↓ lytic concentration and activity in preterms, ↑ with the gestational age	14
Neutrophil storage pool	NB < 32 weeks of gestation have 20% of the neutrophils storage of full term NB	20
Neutrophils endothelial adhesion	↓ L-selectin expression in NB When stimulated, the NB neutrophils produce ↓ CR3 adhesin than the adult Concentration of CR3 in neutrophils increase with the gestational age	25,24 26 26,27
Neutrophils deformability	Decreased in NB in general, not only in pretermatures	27,28
Antimicrobial substances	The concentration of BPI in NB is 3-4 times smaller than in adults, and in pretermatures it is still smaller	29,30
<i>Natural killer cells</i>	Decreased number and function	37
T lymphocytes <i>helper</i> (CD4+)	The function of stimulating B-lymphocytes to produce antibodies is ↓ in full term and preterm NB	37
Cytotoxic lymphocytes (CD8+)	Cytotoxic activity ↓ full term and preterm NB	36
Passive immunity	The concentration of IgG depends on the transplacental transfer and ↑ with gestational age	43

MHC = major histocompatibility antigens; CR3 = complement receptor 3; BPI = bactericidal permeability increasing protein; NB = newborn.

Consequently, the therapeutic use of emollients has been proposed only for the sites with skin lesions. However, no studies have validated their use.

### **Intravenous immunoglobulin**

The use of intravenous immunoglobulin (IVIg) aims to allow IgG to bind to receptors on the cell surface, promote opsonic and antibody-dependent cytotoxic activities, activate the complement and improve neutrophil chemotaxis. After noting that the serum immunoglobulin level is low in extremely preterm infants, the administration of intravenous immunoglobulin from a pool of adult donors would seem logical for the prevention of nosocomial sepsis. Although it has been shown that its administration is safe, its efficacy remains arguable.

The results of several studies assessing the efficacy of the prophylaxis against sepsis in preterm infants through intravenous administration are controversial; some of them suggest benefits while others do not. Baker et al.<sup>49</sup>,

by studying 558 newborns weighing between 500 and 1,250 g, found a lower risk of nosocomial infection and length of hospital stay in those infants who received IVIg (500 mg/kg/day) than among those treated with placebo. Fanaroff et al.<sup>50</sup> carried out a controlled clinical trial that included 2,416 newborns weighing less than 1,500 g who received intravenous doses of 700-900 mg/kg of IVIg or placebo every 14 days until they achieved 1,800 g, but no reduction in the incidence of nosocomial infection, mortality or other outcomes was observed. Usually, the discrepant results are attributed to the variable titers of specific immunoglobulins against the causative agent of nosocomial sepsis.

Recently, Ohlsson et al.<sup>51</sup> have conducted a meta-analysis of the data about 19 placebo-controlled or noninterventional studies with the aim of gathering information on the largest possible number of infants who received intravenous immunoglobulin as prophylaxis during eight or more days, including more than 5,000 newborns weighing less than 2,500 g. A 3% reduction in the

incidence of sepsis and a 4% reduction in the occurrence of one or more episodes of any severe infection were obtained. These results showed that it would be necessary to treat 33 and 25 infants in order to prevent one case of sepsis and one case of any other severe infection, respectively. However, no decrease in mortality was observed, nor in the occurrence of necrotizing enterocolitis, bronchopulmonary dysplasia, intraventricular hemorrhage or in the length of hospital stay.

Currently, the use of formulations containing hyperimmune IgG antibodies against specific antibodies and/or IgM antibodies has also been considered. However, clinical trials are not available yet.<sup>52</sup> It should be underscored that there are no available data justifying the routine administration of intravenous immunoglobulin for the prevention of nosocomial sepsis. This use is justifiable in units with a high incidence of nosocomial infection and in which the incidence remains high even after control measures against infection are strengthened. Nevertheless, the prophylactic use should be based upon a careful assessment of costs and clinical benefits.

#### **Myeloid colony-stimulating factors**

These are hematopoietic growth factors that promote the proliferation, differentiation, maturation, survival and activation of neutrophils and macrophages. Their cord blood levels are correlated with gestational age<sup>21</sup> and the production by mononuclear cells in fetuses and preterm infants is significantly lower than in full-term newborns.<sup>53</sup> Given the potential capacity to improve the phagocytosis of bacteria and fungi and the frequent occurrence of neutropenia and impaired neutrophil function, the application of these recombinant human factors was analyzed in terms of prophylaxis and treatment of neonatal sepsis. Two factors were evaluated in newborn infants: granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF).

It has been well documented that both factors increase the number of circulating neutrophils, increase the bone marrow neutrophil storage pool and increase the expression of neutrophil C3bi receptors, in the absence of short or long-term adverse effects.<sup>16</sup> However, the administration of these factors did not remarkably reduce the incidence of nosocomial sepsis in most extremely preterm infants.<sup>54</sup>

Carr et al.<sup>55</sup> performed a meta-analysis of three controlled studies that aimed to improve immunity and reduce the incidence of nosocomial sepsis and mortality from infection. Altogether, these studies included 359 newborn infants with less than 32 weeks of gestational age or weighing less than 1,000 g, either with or without neutropenia, and who received GM-CSF immediately after birth. Due to the variability of studies, it was not possible to verify whether the administration of GM-CSF reduced the incidence of nosocomial sepsis, but its administration did not cause any decrease in mortality. Therefore, up to the present time, there are not sufficient data to recommend the prophylactic routine administration of GM-CSF for the prevention of nosocomial sepsis.

These stimulators provide protection against infection when given to preterm infants with less than 32 weeks and who are neutropenic ( $< 1,700/\text{mm}^3$ ) or who are at risk for neutropenia in the postnatal period. However, only one controlled study has been conducted with this group of infants,<sup>56</sup> wherein the incidence of systemic infection decreased from 53 to 31%, but without statistical significance, possibly due to the small number of infants analyzed. A similar effect was observed in a non-controlled study in which G-CSF was administered to neutropenic infants.<sup>57</sup> By the end of 2004, a large number of patients will have been recruited for a UK study aimed to verify whether the prophylactic use of GM-CSF is able to reduce systemic infection or mortality in infants at high risk for postnatal neutropenia.

#### **Final remarks**

Although we know the immunologic limitations of preterm infants, the alternatives to intervention have been scarce so far and future studies should explore different combinations of immunoprophylactic measures, including maternal vaccination<sup>58</sup> and newborn infants. Additionally, the measures intended to reduce the virulence of pathogens that cause nosocomial sepsis are still incipient.<sup>59,60</sup> Therefore, a great deal of effort should be made so that preterm infants, especially extremely preterm ones, are not colonized and invaded by nosocomial pathogens, since once the infection is established, the consequences will be potentially deleterious. It is therefore necessary to intervene in factors extrinsic to extremely preterm infants that are strongly associated with the occurrence of nosocomial infections. The necessary measures, among which hand hygiene plays a crucial role,<sup>61</sup> are summarized in Table 4 and reviewed in other articles.<sup>62,63</sup>

**Table 4 -** Potentially better practices to prevent nosocomial infections

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Handwashing	<ul style="list-style-type: none"> <li>- Careful and meticulous handwashing, monitoring and reporting of staff compliance to handwashing practices</li> </ul>
Nutrition	<ul style="list-style-type: none"> <li>- Do not change parenteral solutions after preparation</li> <li>- Start enteral feeding as soon as possible</li> <li>- Reduce exposure to intravenous lipids and parenteral feeding</li> <li>- Promote human milk use, which should be safely collected and stored</li> </ul>
Skin care	<ul style="list-style-type: none"> <li>- Start the skin preparation for all newborns <math>&lt; 1.000</math> g to promote skin maturation and prevent rashes</li> <li>- Reduce lab tests that require ankle and venous punctures</li> <li>- Develop a systematic approach to reduce the frequency and number of skin punctures for venous catheter management</li> </ul>

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(Table 4 continues on the next page)



**Table 4 -** Potentially better practices to prevent nosocomial infections (continuation)

## Respiration

- Reduce intubation days
- Avoid interruption of the endotracheal catheter/ventilator circuit

## Vascular approach

- Reduce the use of central vascular accesses; minimize the frequency of daily inputs and period of use
- Insert vascular access when the need for intravenous therapy is expected to be required for prolonged time
- Create rules and procedures for obtaining and handling venous access; monitor regularly the compliance to these measures and procedures

## Diagnosis

- Set a minimum volume for blood culture sampling (1 ml for each tube of aerobic culture)
- Preferably, collect two samples of 1 ml for each aerobic blood culture
- Develop a method to identify real positive culture contamination

## Habits of the intensive care unit team

- Promote support care with the least manipulation
- Development and maintenance of cooperation and team work that supports and motivates staff to be responsible for prognostics

Adapted from Kilbride.<sup>63</sup>**References**

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