The mucosal immune system protects the most vulnerable surfaces of the body (e.g., the gastrointestinal tract, lungs, and eyes) from the colonization and/or invasion by pathogenic microorganisms. Arguably the most important time at which this system is needed is at birth, when the infant moves out of the sterile environment of the womb. Maternal immunoglobulin G transmitted across the placenta into the fetal circulation will provide some protection, as will IgA in colostrum (assuming the infant is breastfed). However, it is important that the infant rapidly generates its own mucosal immune response against pathogens. Because of this it is important to know the maturity of the mucosal immune system at birth.

Previous studies on the ontogeny of the mucosal immune response were mainly restricted to experimental animals (mostly rodents). The rodent period of gestation is 21 days; in man it is 270 to 280 days and there is no a priori reason to assume that there is equivalent prenatal and postnatal development of the mucosal lymphoid tissues in the two species. The development of monoclonal antibodies and immunohistochemistry now allows us to investigate in detail the cells of the mucosal immune system in situ in man. Functional studies during development are limited, however, due to the relative lack of availability of human fetal intestine and the problems in isolating sufficient cells. Here I shall review recently published studies on the ontogeny of the human mucosal immune system. For ease of discussion the separate compartments of the system, that is, Peyer’s patches, lamina propria, and epithelium, will be discussed separately.

GUT EPITHELIUM

The function of the gut depends crucially on the epithelium, both for absorption and as a barrier. Columnar epithelium and villi develop at 9 to 10 weeks’ gestation in the human fetal gastrointestinal tract (1). Crypt formation begins in the proximal small bowel at 10 to 11 weeks’ gestation and develops distally. Although there is some increase in their levels throughout gestation, brush-border enzymes are present from 10 to 11 weeks’ gestation. Paneth cells are present in crypts at this time and the surface epithelium contains mature goblet cells.
Intraepithelial Lymphocytes

Intraepithelial lymphocytes (IEL) can be identified morphologically in human small bowel at 11 weeks of gestation, at which time there are three lymphocytes for every 1000 epithelial cells (2). Their numbers increase thereafter. These results have been confirmed and extended in more recent immunohistochemical studies (3). At 11 weeks of gestation CD3⁺ IEL can be identified. Some of these are CD4⁺ and some are CD8⁺; however, the numbers are too small to allow reliable determination of the relative proportions of each subset. CD3⁺ IEL increase in number with time (Table 1) and by 17 to 19 weeks there are two to five CD3⁺ IEL per 100 epithelial cells (Fig. 1). The number of CD8⁺ cells exceeds the number of CD4⁺ IEL, as it does in the postnatal bowel. Remarkably, however, about 50% of the IEL are

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>IEL/100 epithelial cells</th>
<th>CD4/CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>11–12</td>
<td>1.1–1.5</td>
<td>Not done</td>
</tr>
<tr>
<td>14–16</td>
<td>1.8–3.2</td>
<td>0.3:1</td>
</tr>
<tr>
<td>17–19</td>
<td>2.3–4.5</td>
<td>0.3:1</td>
</tr>
</tbody>
</table>

IEL, intraepithelial lymphocytes.

FIG. 1. CD3⁺ IEL in 17-week-old fetal human small intestine. Immunoperoxidase, original magnification ×280.
CD4⁻ and CD8⁻ (4). Cells of this phenotype make up about 6% of IEL in postnatal gut (5).

T cell receptor (TCR) expression has also been studied in fetal human small intestine at 14 to 20 weeks' gestation (6). There are virtually no γδ TCR⁺ T cells in fetal IEL between 14 and 17 weeks, but at 18 weeks there is a dramatic rise in their numbers, so that they make up about 30% of the CD3⁺ cells. All of the γδ TCR⁺ IELs use the Vδ2 gene segment. This is the form used by peripheral blood γδ TCR⁺ T cells, but is relatively uncommon in γδ TCR⁺ IEL in normal human small intestine; nearly all of these use the Vδ1 gene segment. It is assumed that the rest of the IELs are α/β T cells, since they are CD3⁺.

In postnatal intestine there is a large population of CD3⁻, CD7⁺ IELs, which are not T cells (5). These cells are absent from fetal intestinal IEL.

HLA-DR EXPRESSION BY EPITHELIAL CELLS

In postnatal small intestine HLA-DR molecules are expressed on villous epithelial cells but are generally absent from crypt epithelial cells and epithelial cells in the large bowel (7,8). Epithelial HLA-DR expression increases in intestinal inflammation, and although epithelial cells can present antigen to T cells (9) it is not yet clear if they do this in vivo. In the fetus up to 17 weeks’ gestation the villous epithelial cells are HLA-DR⁻. At 18 weeks HLA-DR can be seen at the tips of the villi in some specimens and by 22 weeks all of the villus epithelial cells may be HLA-DR positive (10). The reason for the appearance of class II molecules at this time is unknown. Epithelial cells can, however, be induced to express Class II molecules prematurely on both villi and crypts by local T-cell-mediated immune reactions in fetal lamina propria (10).

SECRETORY COMPONENT

Secretory component is synthesized by crypt cells and forms the “lock and key” mechanism by which dimeric IgA is actively transported from the lamina propria, across the epithelium, and into the lumen. Secretory component can be detected by immunofluorescence in epithelial cells of the human fetal gut at 16 weeks’ gestation (11,12). The density of staining in individual cells increases dramatically at around 20 weeks. Interestingly, this is also the time when HLA-DR expression increases. The development of secretory component is unrelated to the presence of IgA plasma cells, which do not appear until after birth (13).

FETAL INTESTINAL LAMINA PROPIA

At the earliest time studied (11 weeks’ gestation), there are virtually no T or B cells in fetal lamina propria. Many of the cells in the lamina propria have macrophage-like morphology, while others are spindle shaped (14). These cells are strongly
CD45\(^+\), HLA-DR\(^+\), HLA-DP\(^+\), CD4\(^+\), and RFD1\(^+\). They are therefore probably macrophages and dendritic cells. The extent of this nonlymphoid infiltrate increases with the age of the fetus. At 11 weeks' gestation there are clusters of HLA-DR\(^+\), CD4\(^+\) cells in the lamina propria, which may be Peyer's patch anlagen (15).

At 14 weeks' gestation small clusters of T and B cells are present in regions of the lamina propria, which are also strongly HLA-DR\(^+\) (15). This is probably the earliest influx of lymphoid cells into the Peyer's patch anlagen described above. B cells are not present outside these clusters in the lamina propria in most fetuses up to 22 weeks' gestation. Plasma cells are also not seen in healthy fetal lamina propria. In fact there are virtually no plasma cells at birth in jejunum, colon, or rectum in the normal healthy human fetal gut (Fig. 2); these do not appear until about 12 days after birth (13). This is presumably a reflection of the lack of antigenic stimulation in the fetus and the increase after birth is probably induced by the appearance of the gut flora. From 14 weeks' gestation, however, there are numerous CD3\(^+\) cells in the lamina propria. Most of these are CD4\(^+\) (3).

PEYER'S PATCHES

Peyer's patches are macroscopically identifiable in fetal human intestine at 24 weeks' gestation (16). Even in the fetus they predominate in the ileum compared to the jejunum or duodenum. The number of Peyer's patches increases with fetal age,
so that at birth there are around 100 patches with more than five follicles in the small intestine.

The first identifiable clusters of T and B cells, forming around clusters of accessory cells, can be seen at 14 weeks' gestation (15). These increase in number and size at 16 weeks' gestation. The T cells are mostly CD4^+ and the B cells are surface IgM^+, IgD^+. There is no cellular zonation. By 19 weeks well-defined Peyer's patches have formed. A striking feature of these is that virtually every cell in the Peyer's patch (including T cells, B cells, and accessory cells) is Class II^+ (Fig. 3). The Peyer's patches contain well-defined, central, B cell zones containing follicular dendritic cells. The follicle B cells are surface IgM^+, IgD^+ and express CD5. Surrounding the follicles are well-defined T cell zones, most of the cells being CD4^+. A follicle-associated epithelium also develops by this time, characterized by a more cuboidal epithelium and a relative sparsity of goblet cells. Putative M cells have been identified in fetal follicle-associated epithelium (1).

There is no evidence of Peyer's patch germinal center formation in healthy fetal human fetal small intestine. After birth, however, these develop rapidly due to antigenic stimulation from luminal bacteria (17).

SALIVARY GLANDS

The salivary glands are important in the defense of the mucosa of the mouth, and there have been several studies on the ontogeny of their immune system. Iwase
et al. (18) reported IgM+ cells in fetal salivary glands at 20 weeks' gestation. These results were confirmed by Thrane et al. (12), who carried out a comprehensive study of the ontogeny of secretory immunity in salivary glands. From 20 weeks' gestation, the occasional salivary gland contains small numbers of cells with cytoplasmic IgA and IgM. The IgA is mostly IgAl and dimeric. After birth there is a dramatic increase in IgA plasma cells at this site.

CONCLUSIONS

In the 8-week period from 11 weeks' to 19 to 20 weeks' gestation humal fetal intestine develops organized Peyer's patches and mucosal T cells in the lamina propria and epithelium. T cell numbers are, however, low compared to postnatal intestine, and the Peyer's patches only contain primary B cell follicles. There is little information on changes that occur between 20 weeks' gestation and birth; however, it would appear that there is little further development of human gut-associated lymphoid tissue until the gut is exposed to food and bacterial antigens. Thus there are no plasma cells in newborn human intestine. It would therefore appear that the mucosal immune system is functionally mature by 20 weeks' gestation, but remains quiescent until birth.

REFERENCES

DISCUSSION

Dr. Boyd: I do not think your notion that the gut is immunologically "virgin" is likely to be secure. We know that the protein content of amniotic fluid reflects maternal plasma composition and not fetal, and that therefore the amniotic fluid is full of proteins that are foreign to the fetal immune system. We also know that proteins are swallowed by the fetus and degraded in the gastrointestinal tract. I therefore suspect that the fetal gut immune system is seeing foreign proteins of maternal origin from mid-gestation onward.

Dr. MacDonald: Although there is no doubt that maternal foreign proteins are present in the amniotic fluid, in the absence of the typical markers of T and B cell activation it is difficult to support the view that any antigenic stimulus is occurring. I am not saying that the antigens are not there, but that there is no evidence of a mucosal immune response in the fetus. We don't see any IgA.

Dr. Guesry: I'm not entirely satisfied with this. Chandra (1) has shown that potent allergens such as eggs, soya, cow's milk, or fish in the maternal diet are capable of stimulating an allergic response in the infant. I accept that there is a similar study from Sweden (2) that shows no effect, but Chandra maintains that this is because the study diet was started too late in the Swedish study. It seems that allergens do find their way into the amniotic fluid and that there are indeed some babies who show IgE-mediated immunological responses on their first contact with foreign antigens, suggesting intrauterine sensitization.

Dr. MacDonald: I have read these papers very critically. I am not satisfied that it has been shown that putting a pregnant woman on a low-allergen diet has any consequences for the baby. However, my own studies have not addressed the question of whether IgE-mediated sensitization does or does not occur in utero.

Dr. Chirico: It should be remembered that there is another mechanism whereby the fetal immune system can be exposed to foreign antigens. This is the anti-idiotypic response of the mother. Anti-idiotypic antibodies can cross the placenta and bring the maternal antigen image to the fetus. This mechanism seems able to prime the fetal immune system in some way.

Dr. MacDonald: Some newborn babies have antibodies to polio virus and this may be the result of an anti-idiotypic response in the mother to polio virus antibodies, an image that the baby recognizes. I think this is very interesting, though in reality it is unlikely to be of great importance. If it were, there would be no point in vaccinating babies. It would only be necessary to vaccinate the mothers. It has been documented in the literature that antibodies can be detected in the serum of neonates who have presumably never been exposed to the relevant antigens, but since the levels of these antibodies are extremely low I question their biological, as opposed to their immunological, significance.

Dr. Simmer: You have avoided discussing the importance of dietary protein as an antigen for the preterm neonate and the issue of its influence on the development of atopy as the
child grows up. This has important significance for neonatologists. If a mother is expressing breast milk to feed her premature infant, do we supplement it with protein-hydrolyzed formula or even with breast milk fortified with protein? Is it important to avoid supplements containing intact cow's milk proteins?

Dr. MacDonald: I am not an expert on this. However, formula-fed preterm babies get fewer allergic reactions and less atopy than full-term babies, though we don't yet understand the reason for this.

Dr. Guesry: There have been many studies published in the last 10 years showing that preterm infants are as capable as full-term babies of becoming sensitized to foreign proteins. When we asked Ann Ferguson for her interpretation of the fact that neonatologists do not seem to see manifestations of food allergy in preterm infants she postulated that the ability to express the clinical manifestations of food allergy was not yet fully developed in such babies. In other words they are sensitized but the T cells are not yet mature enough to express the allergy.

Dr. MacDonald: I find difficulty in visualizing such a phenomenon in immunological terms. Perhaps Dr. Schmitz could clarify this.

Dr. Schmitz: The answer is not easy to get. In a study we did, we were not able to find any modification of the IgA response in preterm infants, and only by feeding them with a formula of low immunogenicity in the first few days of life were we able to modulate the IgG response to antigens.

Dr. MacDonald: But the clinical outcome in the baby was not modified. The genetic component of atopy is in my view the most important factor.

Dr. Bracci: Premature infants may have serious difficulty in activating phagocytes and in superoxide production. Do you think that this deficiency, which may be the most important immunological problem in preterm infants, may play a role in lowering the defenses of the intestinal mucosa? This is important because some treatments, such as steroids, may activate the antioxidant system but also lower superoxide production by phagocytes.

Dr. MacDonald: Data on newborn phagocytic cells are less complete than those on blood cells and mononuclear cells. I accept that macrophages in the gut may be poorly activated. There may be a window of susceptibility here until the gut flora are established. Once the gut is colonized, however, the macrophages will be nonspecifically activated.

Dr. Carapella: I should like to know if the M cell, as an antigen-presenting cell, appears before or after birth.

Dr. MacDonald: The M cell is not an antigen-presenting cell; it is an antigen transport cell. There is only one paper showing the presence of M cells in the fetus, but the biological significance of the M cell is still very uncertain. The limited available evidence suggests that the M cells develop in the fetus before birth and enable antigens to be transported from the gastrointestinal tract.

REFERENCES