Immunohistologic Localization of Immunoglobulins, Secretory Component, and Lactoferrin in the Developing Human Fetus[1] (36188)

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The circulating immunoglobulins in the normal human newborn consist almost entirely of γG immunoglobulin, which is largely acquired by placental transfer from the mother. The newborn seems to respond poorly to immunization with various infectious antigens in the immediate newborn period (1–3). It has been suggested that the human fetus is immunologically incompetent, and deficient in immunocompetent lymphoid elements which support and maintain an immunologic response after birth (4–6). On the other hand other investigations directed towards the pathogenesis of intrauterine infections have clearly demonstrated that the human fetus is able to support variable levels of an immune response following an infectious antigenic stimulus (7–9). Relatively little information is available regarding the distribution and development of the cellular elements of the immune system, especially the secretory system in the developing normal human fetus.

The present investigation was initiated to characterize the distribution of γG, γA, and γM immunoglobulins, secretory component (SC) and lactoferrin (LF) in the developing fetus, employing immunofluorescent staining of lymphoid and epithelial tissues as a morphologic indicator of specific protein production.

Materials and Methods. Tissue specimens. Tissue specimens were obtained from the following groups of fetuses, which were aborted by means of hysterotomies, endocervical dilatation and curettage (D&C) and saline inductions. Group I consisted of five fetuses obtained between 8–12 weeks of gestation. Group II consisted of four fetuses obtained after 13–17 weeks of gestation. Group III comprised of five fetuses aborted after 18–22 weeks of gestation. All abortions were performed for noninfectious therapeutic reasons. All fetuses included in this study were considered to be normal histologically. The fetal age was calculated from the date of last menstrual period of the mother, and correlated with the clinical findings of uterine size and the size of the abortus. Ramdomly selected sections of fetal liver, spleen, lung, kidney, adrenals, stomach, intestine, thymus, heart, placenta, and amniotic membranes were studied for immunoglobulins, SC and LF containing cells.

Preparation of tissues. Tissue specimens were prepared for cryostat sectioning according to the method described by Eidelman and Davis (10). Specimens were fixed in isotonic buffered formaldehyde, followed by an overnight wash in 30% sucrose solution. All tissues were frozen in isopentane. Consecutive sections approximately 4 μ thick were obtained from each tissue. Dried sections were fixed briefly in buffered formalin, washed in phosphate buffered saline (PBS) pH 7.2 and then stained with fluorescein conjugated antisera.

Fluorescein labelled antisera. The antisera employed in this investigation and the preparation thereof have been described in a previous report (11). Rabbit antisera directed against human γG, γA and γM proteins were made specific for their respective heavy (H) chain classes by appropriate absorption to remove light (L) chain antibodies as previously described (12). Antisera to secretory γA was prepared by immunizing goats with purified colostral γA (13). This antiserum

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was absorbed with Cohn fracton II to make it specific for secretory γA, or with normal human serum to make it specific for SC. All secretory γA antiserum preparations were also absorbed with purified LF obtained from human colostrum (12). Antiserum specific for LF was prepared by immunizing rabbits with LF purified from human colostrum as described previously (11).

The specificity of each antiserum was tested by immunoelectrophoresis and Ouchterlony analysis. All antisera labelled with fluorescein isothiocyanate (FITC) had a molar F/p ratio of 3–4 and were employed at a dilution which provided ¼–½ units of activity according to the methods reported by Beutner *et al.* (14). All FITC labelled antisera were absorbed with rabbit liver powder and human ABO red blood cells before use.

Immunohistological studies. Tissue specimens were processed according to previously described methods (11). Consecutive sections of the varous tissues were stained with the five antisera. Fluorescence blocking experiments included absorption of the various antisera with purified preparations of γA, γM, and Cohn fraction II for γG. Controls for SC staining included absorption of this antiserum with purified preparations of SC and secretory γA. Staining with anti LF was specially blocked with purified LF obtained from human colostrum. All slides were examined immediately with a Leitz ortholux microscope equipped with an osram HBO 200 W high pressure mercury vapor lamp, UGI exiter filter, and a BG 38 suppression filter. Photographs were taken on Kodak high speed film.

Results. Distribution of immunoglobulins SC and LF in 8–12 week old fetuses. As shown in Table I no immunofluorescence was detected against γA, γM immunoglobulins, and LF in any of the specimens tested, except in an occasional polymorphonuclear leukocyte contained in mesentery of an eight week fetus. γG staining was detected in sections of lung, placenta and amniotic membrane, except in the two earliest (eight week) fetuses which were completely negative for any immunoglobulin. The γG staining was observed just below the basement membrane of the bronchial epithelium in the cytotrophoblast of the placenta, and the reticular layer of the amniotic membrane. No γG staining was detected in any of the remaining tissues studied (Table I).

TABLE I. Distribution and Localization of Immunoglobulins, Secretory Component and Lactoferrin in Different Tissues of the Developing Fetus at Various Gestational Ages.

	Gestational age in weeks		
	8–12	13–17	18–22
Thymus	—[a]	—	γG,LF[d]
Liver	—	—	γG,LF[d]
Spleen	—	—	γG
Lung	SC[b],γG[c]	SC,γG	SC,γG
G.I.T.	LF[d]	SC	SC,γG
Kidney	—	SC,γG	SC,γG
Adrenal	—	—	γG
Placenta	γG	γG,LF[d]	γG,LF[d]
Membrane	γG	SC,γG	SC,γG,LF[d]

[a] No detectable staining.

[b] Secretory component staining on surface of mucosa, intra- and interepithelial cell staining. See text for details.

[c] Interstitial staining only.

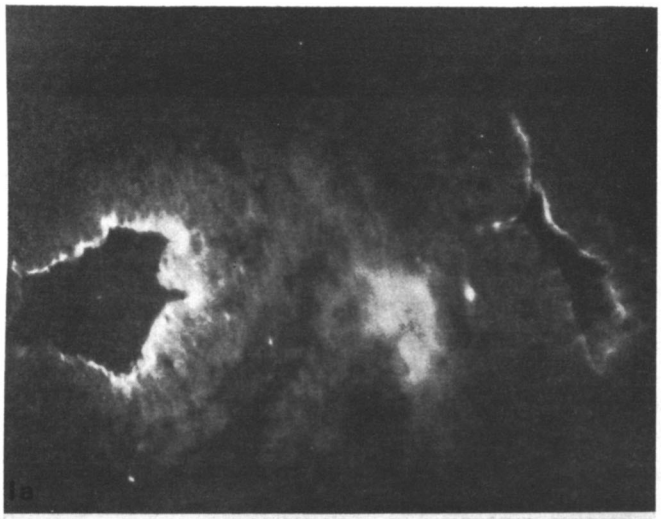
[d] Lactoferrin in polymorphonuclear leukocytes only.

SC was detected in the bronchial surface epithelium and in the intercellular regions between the epithelial cells. Staining patterns for SC were consistent in all specimens tested including the two eight week fetuses with no detectable immunoglobulins. No SC staining was observed in any other tissues of 8–12 week old fetuses. Figure 1a illustrates a representative immunofluorescent pattern of lung of an 8-week old fetus.

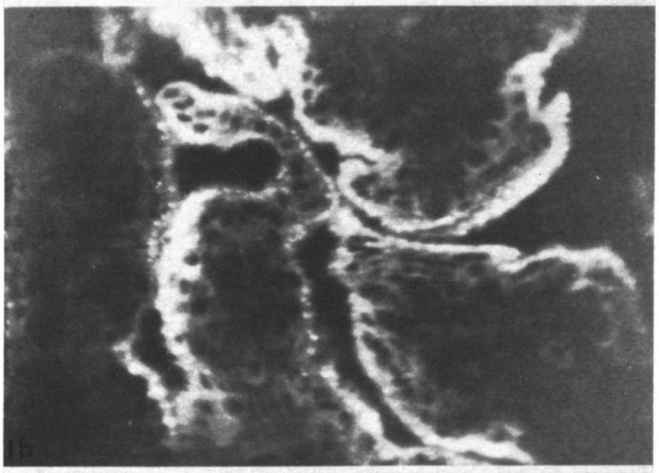
Distribution of immunoglobulins, SC and LF in 13–17 week old fetuses. γA and γM immunoglobulin staining was not observed in any of the tissues studied. Occasional polymorphonuclear staining for LF was observed in the placenta (Table I).

No γG was detected in the specimens of thymus, liver, spleen, stomach, intestine and adrenals. However, γG was readily detected in lung, kidney, placenta and amniotic membrane (Table I). The morphologic localization of γG immunoglobulin in 13–17 week fetuses was similar to that observed in 8–12 week old fetuses.

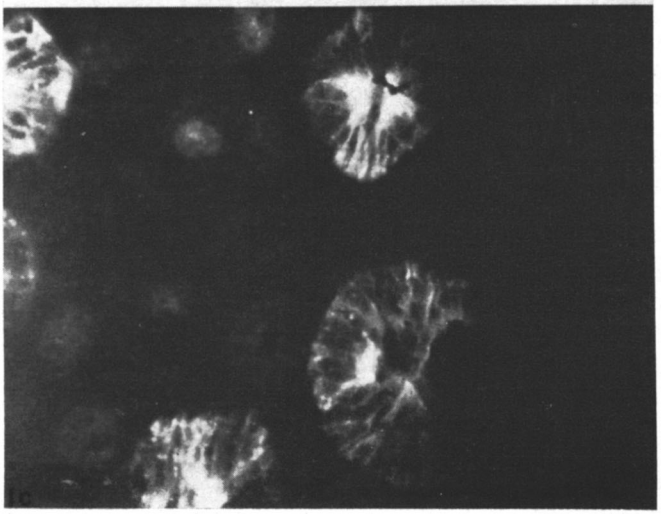
SC was regularly localized in the surface and intercellular regions of the tubular epithelium of the kidneys, surface and interepithe-



1a



1b



1c

lium of bronchi and stomach mucosa, and on the surface of the amnion. No SC staining was demonstrable in thymus, liver, adrenal and small bowel. Figure 1b illustrates a representative pattern of placenta of a 12.5 week fetus.

Distribution of immunoglobulins SC and LF in 18–22 week old fetuses. Staining for γA or γM immunoglobulin could not be detected in 18–22 week old fetuses. However, polymorphonuclear staining for LF was observed frequently in the specimens of 18–22 week fetuses. Detectable staining for LF was observed in polymorphonuclear leukocytes in thymus, liver, placenta and membranes. No LF was detected in specimens of spleen, intestine, lung, kidney and adrenals (Table I).

Significant γG was observed for the first time in the majority of tissues in 18–22 week old fetuses. γG immunoglobulin was regularly detected in the interstitial area of the liver, small bowel, lung, kidney, placenta and amniotic membranes. However, little γG was seen in the thymus, spleen and adrenals (Table I).

Staining for SC also appeared to be present in a greater number of tissues of 18–22 week old fetuses. SC was regularly detected on the surface of and within epithelial cells of the small bowel, lung, kidney, and amnion. The distribution of SC staining in 18–22 week old fetuses was quite similar to that observed in 8–17 week old fetuses. No SC was detected in the liver, spleen, thymus, adrenal and placental tissues studied (Table I and Fig. 1c).

Discussion. The observations presented in this paper indicate that although γG is present in the interstitium of various organs in the first 22 weeks of fetal life, cells containing immunoglobulins cannot be identified using the fluorescent antibody technique. In this respect this study is similar to the earlier work of Bridges *et al.* (6) and Black and Speir (15) who have also reported the absence of detectable plasma cell staining in the normal human fetus. Two fetuses obtained at eight weeks of gestation showed no immunoglobulin staining in any tissues, suggesting that there is no significant placental transfer of γG from mother to fetus by eight weeks of gestation. However, free lying (interstitial) γG was found in the bronchial region at about ten weeks, and subsequently, in other tissues.

Several reports (16–19) have suggested that some immunoglobulin synthesis occurs in the normal fetus during the first trimester. Gitlin *et al.* (19) using incorporation of C_{14} labeled amino acids in tissue cultures demonstrated γG production by liver and gastrointestinal tract as early as 12 weeks of gestation and γM production at 10.5 weeks. The differences between the study of Gitlin *et al.* (19) and the present one may be a result of sensitivity. It seems likely that so few immunoglobulin producing cells are present in the fetus that they are undetectable by the fluorescent antibody method. That the fetus is potentially immunocompetent is also suggested by the fetal response to an infectious antigenic stimulus which has been demonstrated repeatedly (7–9). Recent studies have also suggested that elevated levels of γM and γA in cord blood occur after intrauterine infections (9) and that these immunoglobulins are of fetal origin. It seems, therefore, that the human fetus is immunologically competent, that a low level of synthesis of immunoglobulins occurs normally, and that this can be markedly increased by antigenic stimuli.

Although secretory component was observed only in the lungs of 8–12 week fetuses, its presence was regularly demonstrated in almost all tissues by 13–22 weeks. The role SC may play in interuterine development of the immune system is not known. Its presence in the earliest fetus, even at 8 weeks, is interesting in view of the observation that γA, with which SC is generally associated, is conspicuously absent during interuterine development. It has been postulated on the basis of *in vitro* culture studies that the synthesis of SC is stimulated by contact with the heavy chains of γA

FIG. 1. a) Section from a lung of an 8-week-old fetus stained with fluorescein labelled SC antiserum showing surface staining of bronchial epithelium. b) Section from a placenta of a 12.5-week-old fetus stained with fluorescein labelled anti-γG antiserum. Note the staining of syncytiotrophoblast. c) Section from kidney of an 18-week-old fetus stained with fluorescein labelled SC antiserum demonstrating intraepithelial and intercellular staining patterns of kidney tubular epithelium.

(20). This may indeed be the case but the present studies clearly demonstrate that SC can be synthesized in the absence of demonstrable γA. This is consistent with the observations that SC is found in nearly normal amounts in the secretions of individuals with agammaglobulinemia or a selective deficiency in γA (12).

Lactoferrin has been observed in many external secretions of the adult. Its precise biological function is at present unknown (21). It has been suggested that lysozyme and lactoferrin may play a role in protection against infectious agents (22). This hypothesis is based on the similar tissue localization of lysozyme and lactoferrin in the granules of polymorphonuclear leukocytes as well as certain epithelial cells, and the bacteriostatic effects of lactoferrin on certain bacteria which require iron for metabolism. Except for an occasional polymorphonuclear leukocyte in the placenta, lactoferrin was not found regularly in fetal tissues until the second trimester. It is interesting that while lactoferrin containing polymorphonuclear leukocytes were frequently found in older fetuses, certain epithelial cells such as those in the salivary gland and kidney which normally contain this protein in the adult, did not stain for lactoferrin. This finding may be due to technical problems such as the difficulty in detecting low levels of lactoferrin distributed throughout large epithelial compared with polymorphonuclear leukocytes where it is confined to small granules. Alternately identical lactoferrin genes in polymorphonuclear leukocytes and epithelial cells may be "turned on" at different times during development or there may be different structural genes producing lactoferrin in these two cell types.

Summary. Immunohistological localization of γG, γA, γM, secretory component (SC) and lactoferrin (LF) in various tissues of 8–22 week human fetuses was studied, using direct immunofluorescent staining technique.

No immunoglobulin-containing cells have been detected in various tissues studied. However, SC was found in the lungs of the earliest fetuses studied (8 weeks) and is readily recognized in most secretory tissues by 18–22 weeks of gestation. The role SC may play in the intrauterine development of the immune system is not known but it is present at a time when immunoglobulins (specifically γA) are not detectable.

Lactoferrin was found regularly in the older fetuses (18–22 weeks) only in polymorphonuclear leukocytes. Epithelial cells such as those of the kidney tubules which contain this protein in the adult, were apparently not synthesizing lactoferrin in sufficient quantity to be detectable by the fluorescent antibody method.

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