

Development of immune response markers in the trachea in the fetal period and the first year of life

Stoltenberg L, Thrane PS, Rognum TO. Development of immune response markers in the trachea in the fetal period and the first year of life.

Immunoglobulin (Ig)-producing cells, T cells (CD3) and epithelial expression of secretory component (SC) and HLA class II determinants (DR, -DP, -DQ) were studied by immunohistochemistry in 16 fetal and 15 postnatal specimens from the tracheal wall. Small amounts of secretory component (SC) was present in the tracheal surface and gland epithelium in the fetal period and increased towards term. A few IgM-, IgD- and IgG-producing cells were present in some fetal specimens but no IgA- and IgE-producing cells were found. Only very few CD3+ T-cells were present in fetal specimens and intraepithelial T-cells were virtually absent until after birth. Premature infants that lived for 1 week had less SC epithelial expression than mature infants of the same age. The density of CD3+ T-cells, IgA-, IgM-producing cells as well as the epithelial SC expression increased rapidly after birth. Epithelial MHC class II expression was absent in fetal specimens. HLA-DR was detected on the apical border of the surface epithelium one week after birth and was extensively expressed throughout the remaining postnatal period. Epithelial DP and PQ expression were virtually absent during this same period. These features probably reflect local activation of the immune system in response to environmental factors.

**L. Stoltenberg,^{1,2} P.S. Thrane,³
T.O. Rognum²**

¹Department of Pediatric Research, ²Institute of Forensic Medicine, ³Medical Faculty, Laboratory for Immunohistochemistry and Immunopathology (LIIPAT), Institute of Pathology, University of Oslo, Rikshospitalet, Norway.

Lauritz Stoltenberg, Department of Pediatric Research, The National Hospital, N-0027 Oslo 1, Norway.

Key words: ontogenesis; mucosal immunity; tracheal wall; immunoglobulin production; secretory component; HLA-DR; T-cells; interferon; tumour necrosis factor.

Received June 30, 1992

Accepted September 10, 1992

Adaption of the fetal immune system is of great importance since it enables the fetus to survive in its maternal host. The placental barrier is not impermeable and allows for the transfer of certain soluble antigens and antibodies (1), T-cell activity has moreover, been demonstrated in cord blood (2). After birth, maturation of a fully functional mucosal immune system is important in order to cope with infections during the postnatal period and through infancy. Transmembrane secretory component (SC) belongs to the Ig supergene family (3) and functions as a transport receptor of J-chain-containing polymeric immunoglobulins (pIg) of the A and M classes (4, 5). It has recently been shown that certain cytokines like gamma-interferon (IFN- γ) (6), tumour necrosis factor-alpha (TNF- α) (7) and interleukin-4 (IL-4) (8) can enhance SC expression. Furthermore, it is now well established that various cytokines (IFN- γ in particular) can modulate major histocompatibility

complex (MHC) class II (HLA-DR, -DP, -DQ) expression (9).

The purpose of this study was to investigate the early development of the mucosal immune system in the upper respiratory tract. There is, to our knowledge, only one previous study of the bronchial mucosal membrane (10), and studies from lung, lung lavage fluid and tracheal aspirate fluid (11–13) show discrepant results.

We have in a previous study shown that the components of the secretory immune system is present in the duodenal mucosa after the second postnatal week and HLA-DR is expressed on the villous epithelium after the first week (14).

We and others have recently been able to show that sudden infant death syndrome (SIDS) victims have a stimulated local immune system in the trachea (15) as well as in other secretory sites (15–17). Normal baseline information is therefore important.

Table 1. Clinicopathological information about human material

Specimens	Gestational period	Sex	Cause of death
1	24 weeks	F	RDS
2	26 weeks	M	Premature/Twin
3	27 weeks	F	RSD*
4	28 weeks	F	Anoxic cerebral damage
5	29 weeks	M	Placental separation*
6	30 weeks	M	RDS*
7	30 weeks	F	RDS**
8	31 weeks	M	Intrauterine death
9	32 weeks	F	Intrauterine death
10	32 weeks	F	Placental separation
11	33 weeks	M	Septicemia
12	35 weeks	M	Hydrocephalus
13	35 weeks	F	Multiple malformations**
14	36 weeks	F	Hydrops fetalis
15	37 weeks	M	Multiple malformations
16	38 weeks	M	Brain malformations
<i>Postnatal period</i>			
17	1 week	F	Hypoplastic aortic arch
18	1 week	F	Congenital vitium*
19	1 week	M	Anoxic cerebral damage*
20	2 weeks	M	Congenital vitium
21	3 weeks	M	Arthrogyrosis
22	1 month	M	Head injury
23	2 months	M	Accident (Anasthetics) per op.
24	4 months	F	Neuroblastoma
25	4 months	M	Pierre Robin/Aspiration
26	5 months	M	Cong. vit./Sudden death.
27	6 months	F	Myocarditis
28	7 months	M	Aspiration
29	8 months	M	Drowning
30	11 months	M	CO-intoxication
31	12 months	M	Infanticide

RDS: Respiratory distress syndrome

* Lived for 1 week after birth.

* Two premature and 2 mature infants that lived for approximately 1 week (5, 6, 7 and 9 days). Examined exclusively with regard to SC and HLA-DR.

Patients and methods

Ten premature fetuses and infants who were either stillborn or died shortly after delivery, 3 premature babies who lived for 7–10 days, 5 full-term neonates who died between the time of birth and 4 weeks thereafter, and 9 infants between 4 months and 1 year, were investigated. Two premature and 2 mature infants were included in this study exclusively with regard to SC and HLA-DR. Clinicopathological information about the subjects is given in Table 1.

Tissue

Tissue specimens were obtained from the tracheal wall at the level of carina during the post-mortem examination (Mean post-mortem time: 28 h, range 8–62 h). Sections approximately 1 mm thick, were paraffin-embedded after fixation in cold ethanol, either directly or after extraction in cold phosphate

buffered saline (PBS) for 24 h (18) at 4°C, to remove extracellular diffusible immunoglobulins.

Immunohistochemistry

Adjacent tissue sections were cut at 6 µm and investigated with direct immunohistochemical technique for tracheal wall expression of secretory component (SC) and MHC class II determinants (HLA-DR, -DP, -DQ), and the presence of IgA-, IgM-, IgG-, IgD- and IgE-producing plasma cells and T-cells (19, 20). A sheep IgG anti SC and polyclonal rabbit reagents with specificities for human (IgG) γ-chain, (IgA) α-chain, IgM µ-chain, IgD δ-chain and IgE ε-chain respectively were all purchased from DAKO immuno-globulins A/S, Denmark. Information about the polyclonal antibodies are given in Table 2. The antibody reagents were applied in various pairs of contrasting colours. In addition, a murine monoclonal anti IgE antibody was applied with a FITC-labelled horse anti-murine antibody in the second layer. Monoclonal antibodies against MHC class II (HLA-DR, -DP and -DQ) determinants and CD3 were purchased from SANBIO, 5400 AM Uden, Holland and applied in combination with a polyclonal anti-serum against epidermal keratin using an avidin-biotin enhanced system as described previously (21). One section was stained by a routine method (HAS) containing haematoxylin, azofloxine and saffron (22).

Fluorescence microscopy and photography

Observations were performed in a Leitz Aristoplan fluorescence microscope. Narrow band excitation and selective filtration of the fluorescence colours were obtained with a Phloem-type epilluminator.

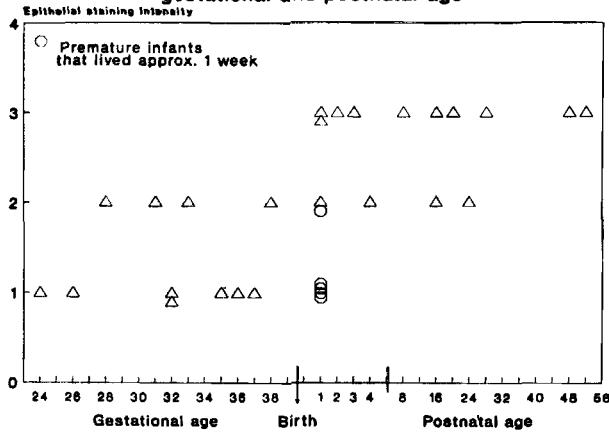
The density of IgA-, IgM-, IgG-, IgD- and IgE-plasma cells were determined by means of an

Table 2. Information about the polyclonal antibodies

Ig	Code	Chain	Product	Working dil. Conjugated
IgA	F204	α-chain	Rabbit anti-human	1:160 FITC-conjugated
IgM	R152	µ-chain	Rabbit anti-human	1:80 TRITC-conjugated
IgG	R151	γ-chain	Rabbit anti-human	1:80 TRITC-conjugated
IgD	F170	δ-chain	Rabbit anti-human	1:5 FITC-conjugated

The specificity and performance of the antibodies have been ascertained in immunohistochemistry as well as indirect ELISA and immunoblotting. Additionally, the specificity has been tested by crossed immunoelectrophoresis using 12.5 µl per square cm gel area against 2 µl human plasma.

SC expression in tracheal wall according to gestational and postnatal age



IgA expression in tracheal wall according to gestational and postnatal age

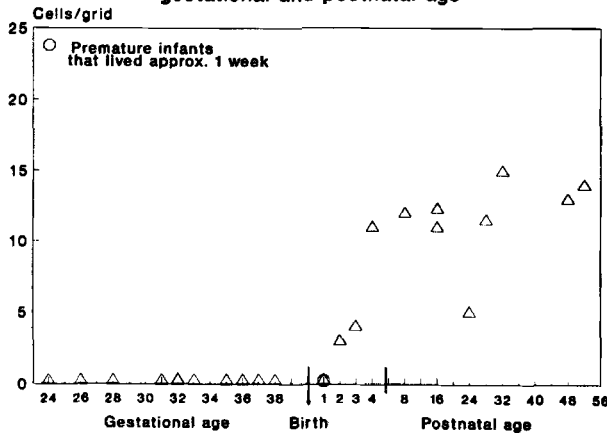


Fig. 1. Epithelial immunofluorescence scores of secretory component (SC) and IgA. SC is present in small amounts from the 24th week of gestation and increases from 30–31 week with intense expression from the 1st–4th week of postnatal life. No IgA immunocytes are present until the 2nd–3rd week of life after which a steady increase is observed throughout the remaining period investigated.

ocular grid (Leitz code No 519902) and given as cell number per grid area (0.1 mm^2). Cell counting was carried out in a systemic manner throughout every tissue section. Both superficial areas and deeper glandular areas were covered. Areas with compact fat and connective tissue devoid of epithelial elements were omitted. The number of tissue units evaluated varied from 16 to 48 depending on the quality of the specimen. Average number of tissue units evaluated was 29. Twenty tissue units were necessary to obtain a stable mean. All fluorescent cells with a discernable nucleus and cell-like body with a pure green or red cytoplasmic colour were included. Staining for SC was scored semiquantitatively on a scale from 0 to 3+. A score of 0 was given for virtually no staining, whereas a score of 3+ indicates a normal staining pattern as seen in older children and adults.

Epithelial staining for HLA class II determinants was also scored from 0 (virtually no staining) to 3+ (intense overall staining).

Agfa 800 ASA daylight film was used in recording the findings. (Both single and double exposures.)

Both the intra- and interpersonal reproducibility of the scoring system was tested blindly.

Statistics

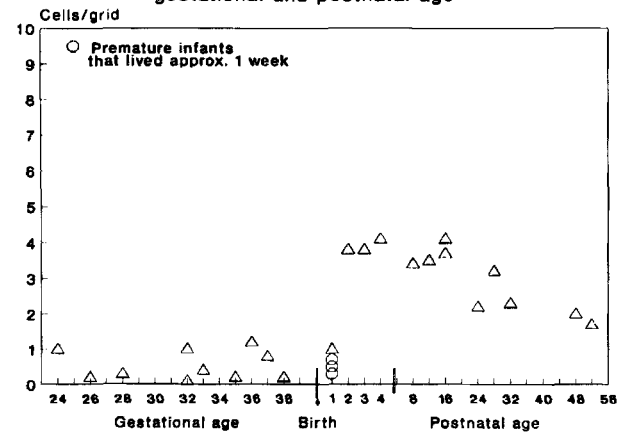
For comparison between groups, the Mann-Whitney U-test was applied and for reproducibility testing, the two sided signtest was used.

Results

Reproducibility

Cell counting by two different observers (L.S. and T.O.R.) was significantly correlated ($r = 0.96$, $p < 0.001$). The blind reproducibility test by the

IgM expression in tracheal wall according to gestational and postnatal age



IgG expression in tracheal wall according to gestational and postnatal age

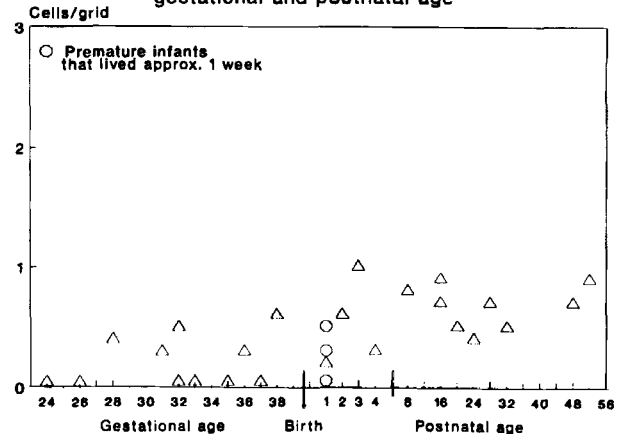


Fig. 2. Epithelial immunofluorescence scores of IgM and IgG. A few IgM immunocytes is observed from the 24th week of gestation with a slight increase after birth. A decrease can be noted at 5–6 months of age. Scattered IgG immunocytes are present throughout the period investigated.

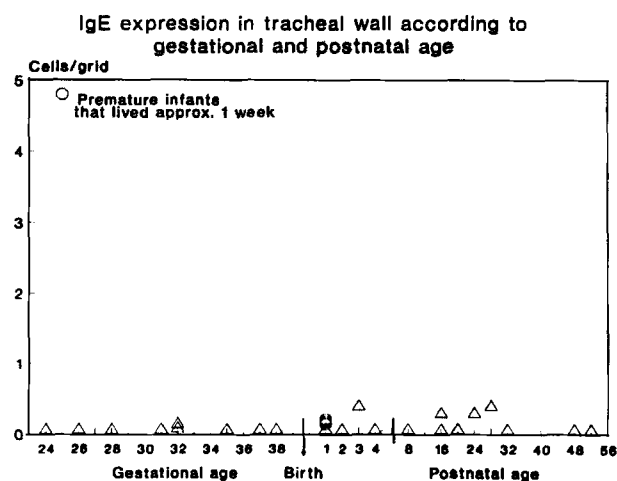
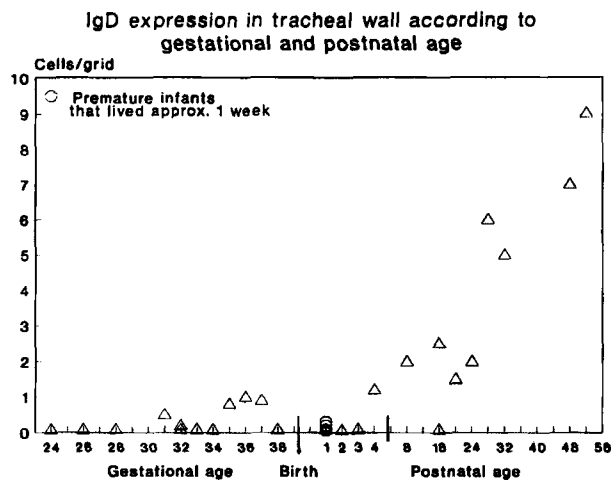


Fig. 3. Epithelial immunofluorescence scores of IgD and IgE. A few B-cells with membrane bound IgD are seen from the 31st week of gestation with a slow increase after birth and a further increase at the end of the 1st year of life. No IgE is observed until the third postnatal week after which a few IgE are present in some of the investigated infants.

same observer (L.S.) was also significantly correlated ($r = 0.97$, $p < 0.001$).

Secretory component (SC)

SC was present in small amounts in both tracheal glandular epithelium and tracheal surface epithelium at the 24th week of gestation and increased from the 30–31 week of gestation and showed intense expression from the 1st–4th week of postnatal life. The premature infants that lived for 7–10 days had less SC-expression than term infants of the same postnatal age (Fig. 1).

IgA-, IgM-, IgG-, IgD- and IgE-plasma cells

No IgA-producing cells was observed until the 2nd–3rd week of life. A steady increase was observed for the next 6–8 weeks with a further slight

increase throughout the remaining period investigated (Fig. 1). A few IgM immunocytes were present from the 24th week of gestation. A slight increase in IgM immunocytes was observed after birth with a slight decrease at 5–6 months of age (Fig. 2). Scattered IgG-producing immunocytes were present throughout the period investigated (Fig. 2). A few B-cells with membrane bound IgD were seen from 31st week of gestation and after birth a slow increase could be observed with a further increase at the end of the 1st year of life (Fig. 3). No IgE was observed until the third postnatal week after which a few IgE were present in some of the investigated infants (Fig. 3).

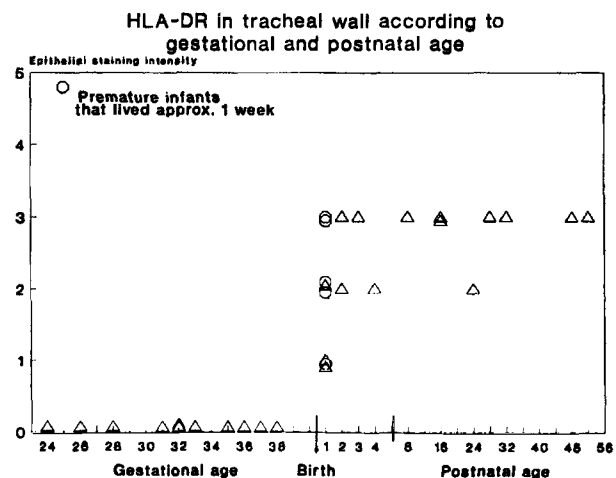
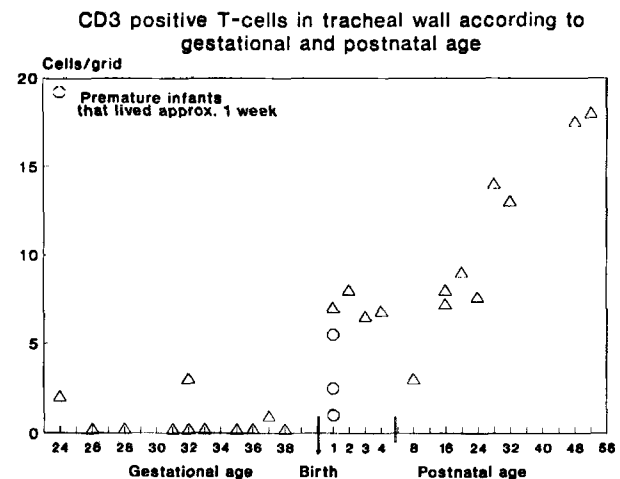


Fig. 4. Epithelial immunofluorescence scores of HLA-DR determinants and CD3+ T-cells. HLA-DR is observed after 1 week of postnatal life and the surface epithelium remains strongly HLA-DR positive throughout the remaining period investigated. CD3+ T-cells are seen immediately after birth and a further increase is observed at 6–7 months of age.

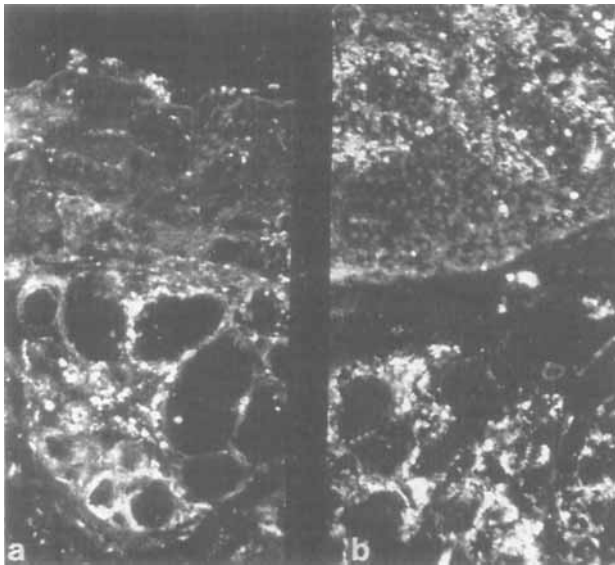


Fig. 5. Immunofluorescence staining of CD3+ T-cells in tracheal lamina propria. a) Infant 4 weeks old. b) Infant 52 weeks old.

CD3+ T cells and epithelial MHC class II (HLA-DR, -DP, -DQ) determinants

CD3+ T cells were detected in the tracheal epithelium and lamina propria immediately after birth and increased rapidly during the first week of life. A further increase could be detected at 6–7 months of age (Fig. 4). HLA-DR could be detected in the surface epithelium after 1 week of postnatal life and the surface epithelium was strongly HLA-DR positive throughout the remaining period investigated. DR-positivity was located mainly in the apical part of the epithelium (Fig. 4). Epithelial DP and DQ expression were absent throughout the entire period investigated.

Discussion

Secretory component was present throughout the period investigated. This confirms a previous study that have demonstrated the presence of SC in apical cytoplasm of columnar epithelium and in ciliated and nonciliated epithelial cells in segmental bronchi near the openings of the ducts from the 16th week of gestation (23). The presence of SC in the tracheal epithelium precedes the appearance of local IgA immunoglobulin-producing cells by several months suggesting that SC-production is independent of the local production of IgA immunoglobulin-producing cells. Similar findings have been demonstrated previously (24). The 5 premature infants (27, 29, 30, 30 and 35 weeks of gestation) that lived for approximately 1 week, had all significantly less SC-expression than mature infants with the same postnatal age ($p < 0.01$). This

effect by prematurity on secretory component expression, is to our knowledge a new observation and will be investigated further in a later study. However, it has previously been shown that prematurity (in particular infants < 33 weeks) effects the production of all the main immunoglobulin groups (25, 26). This may in part explain why infections are a major problem in neonates less than 33 weeks of gestation.

The absence of epithelial MHC class II (HLA-DR, -DP, -DQ) in fetal tracheal epithelium, corresponds with recent studies in duodenal mucosa (14). HLA-DR expression was observed 1 week after delivery. The staining intensity increased from the 1st to the 2nd week of life and maintained the same intensity throughout the period investigated. This is in agreement with the study of Daar et al. (27) but in contrast to that of Natali et al. (28) who concluded that a fully developed tissue distribution of HLA-DR antigen was seen already as early as in the 26th gestational week. Our study indicate that antigenic and mitogenic stimuli are required for induction of epithelial HLA-DR determinants.

The density of CD3+ T cells increased shortly after birth. This rapid increase at the time of birth has previously been observed (26). A further increase was observed at 20–24 weeks of age. It is known from clinical experience that young infants are more susceptible to serious infections, predominantly viral infections. This might be of interest since a fully developed T-cell system is required for a proper defence against viruses. Clinically it is most evident in cases of varicella in the newborn period and perinatal cytomeglo-virus (CMV) infections (29).

The concomitant rise in SC, HLA-DR and CD3+ T-cells suggests that T cell derived lymphokines are important in the induction of both SC and DR expression (6–9).

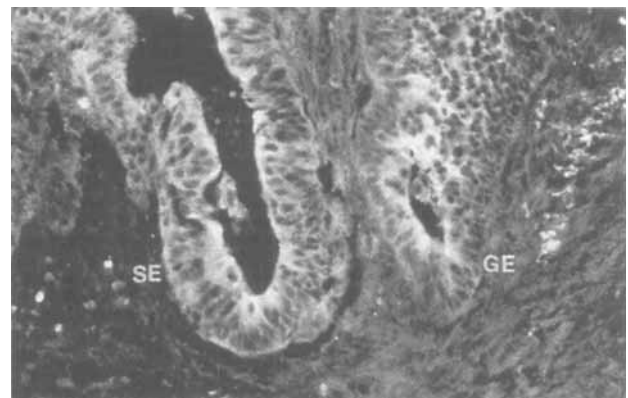


Fig. 6. Immunofluorescence staining of secretory component (SC) in an infant 2 weeks old. It is present in both tracheal surface epithelium (SE) and tracheal gland epithelium (GE). The staining intensity is as seen in older infants and adults.

IgA producing cells were first observed 2 weeks after birth and reached a stable number at approximately 2 months of postnatal age, which is comparable to findings in salivary glands and the gut. IgG was not seen until the 28th gestational week. IgM producing cells were seen as early as the 24th gestational week, and this finding is in agreement with that of Iwase et al. (23) but in contrast to that of Takamura et al. (10) who was unable to detect IgM immunocytes until one month after birth. The density of IgM-producing plasma cells increased steadily until 6–7 month of age when a slight decrease was observed thereafter. This shift from predominantly IgM-producing cells to IgA-producing cells may reflect an increase in antigenic burden during this period that may saturate the secretory component transport system. It is known that this SC-dependant transmembranous transport-system has a higher affinity for the IgA than IgM (30).

One might question that infants with multiple malformations might have a low expression of some or all of the markers studied. However, these infants had comparable immunoglobulin expression in their trachea compared to age-matched infants with other causes of death. Athrogyphosis, although a heterogenous group, is not known to be associated with defects in the immune system (31). Pierre Robin syndrome, consisting of micrognathia with glossoptosis and high arched or cleft palate, is a facial growth disorder with no associated immunodeficiency.

In conclusion, SC-production seems to be independent of the local production of IgA immunoglobulin-producing cells. Five premature infants that lived for approximately 1 week, had all significantly less ($p < 0.01$) SC-expression than mature infants of the same postnatal age. Furthermore, antigenic and/or mitogenic stimuli seem to be required for the induction of epithelial IgA and HLA-DR determinants. A rapid increase in CD3+ T-cells was observed shortly after birth. The concomitant rise in SC, HLA-DR and CD3+ T-cells, suggests that T-cells derived cytokines may be responsible for the induction of both SC and HLA-DR.

Acknowledgements

Supported by the Norwegian Research Council for Science and the Humanities, The Norwegian Red Cross and Grieg Ltd.

References

1. SIEGEL I, GLEICHER N. Development of the fetal immune system. In: Reproductive immunology. New York: Liss, 1981: 31–40.
2. OLDSTONE MBA, THESIN A, MORETTA L. Active thymus derived suppressor lymphocytes in human cord blood. *Nature* 1977; 269: 333–5.
3. KRAJCI P, SOLBERG R, SANDBERG M, ØYEN O, JAHNSEN T, BRANDTZAEG P. Molecular cloning of the human transmembrane secretory component (poly Ig receptor) and its mRNA expression in human tissues. *Bioch Bioph Res Comm* 1989; 3: 783–9.
4. BRANDTZAEG P, PRYDTZ H. Direct evidence for an integrated function of J-chain and secretory component in epithelial transport of immunoglobulin. *Nature* 1984; 311: 71–3.
5. BRANDTZAEG P. Role of J-chain and secretory component in receptor-mediated glandular and hepatic transport of immunoglobulins in man. *Scand J Immunol* 1985; 22: 111–46.
6. SOLLIED LM, KVALE P, BRANDTZAEG P, MARKUSSEN G, THORSBY E. Interferon enhances expression of secretory component, the epithelial receptor for polymeric immunoglobulins. *J Immunol* 1987; 138: 1–4.
7. KVALE D, LØVHAUG D, SOLLID M, BRANDTZAEG P. Tumor necrosis factor- α upregulates expression of secretory component, the epithelial receptor for polymeric immunoglobulins. *J Immunol* 1988; 140: 3086–90.
8. PHILLIPS JO, EVERSON MP, MOLDOVEANU Z, LUE C, MESTECKY J. Synergistic effect of IL-4 and IFN- γ on the expression of polymeric Ig receptor (secretory component) and IgA binding by Human epithelial cells. *J Immunol* 1990; 6: 1–5.
9. BASHAM T, SMITH W, LANIER L, MORHENN W, MARIGAN T. Regulation of expression of Class II major histocompatibility antigens on human peripheral blood monocytes and Langerhans cells by interferon. *Hum Immunol* 1984; 10: 83–7.
10. TAKAMURA T, EISHI Y. Distribution of secretory component and immunoglobulins in the developing lung. *Am Rev Respir Dis* 1985; 131: 125–30.
11. FORSYTH K, KOH L, LAWRENCE A, BRADLEY J. Immunoglobulin profile of tracheal aspirate fluid in intubated children. *J Immunol* 1988; 71: 357–61.
12. SENHAUSER F, BALLOCH A, SHELTON M, DOYLE L, YU V, ROBERTSON D. A longitudinal study of immunoglobulin and antibody concentrations in lower respiratory tract secretions from very premature neonates. In: *Adv mucosal immunol*. MacDonald TT, et al., eds. Dordrecht: Kluwer, 1989: 459–60.
13. MORO I, IWASE T, KOMIYMA K, et al. Distribution of IgG subclasses in human fetus. In: MacDonald, et al., eds. *Adv mucosal immunol*. Dordrecht: Kluwer, 1989: 459–60.
14. ROGNUM TO, THRANE PS, STOLTENBERG L, VEGE Å, BRANDTZAEG P. Development of intestinal immunity in fetal life and the first months after birth. *Pediatr Res* (in press).
15. STOLTENBERG L, SAUGSTAD OD, ROGNUM TO. SIDS victims show local IgM response in tracheal wall and IgA response in duodenal mucosa. *Pediatr Res* 1992; 31: 372–5.
16. THRANE PS, ROGNUM TO, BRANDTZAEG P. Sudden infant death syndrome (SIDS): increased immune response in upper respiratory and digestive tracts in SIDS. *Lancet* 1990; 335: 229–30.
17. FORSYTH K, WEEKS SC, SKINNER J, BRADLEY J. Lung immunoglobulins in the sudden infant death syndrome. *Br Med J* 1989; 298: 23–6.
18. BRANDTZAEG P. Mucosal and glandular distribution of immunoglobulin components. *Immunohistochemistry with a cold ethanol-fixation technique*. *Immunology* 1974; 26: 731–7.

19. BRANDTZAEG P, BAKLIEN K, FAUSA O, HOEL PS. Immunohistochemical characterization of local immunoglobulin formation in ulcerative colitis. *Gastroenterology* 1974; 66: 1123-36.
20. ROGNUM TO, BRANDTZAEG P, BAKLIEN K, HOGNESTAD J. Immunoglobulin-producing cells in the "transitional" mucosa adjacent to adenocarcinomas of human large bowel. *Int J Cancer* 1979; 23: 165-73.
21. BRANDTZAEG P, ROGNUM TO. Evaluation of tissue preparations methods and paired immunofluorescence staining for immunocytochemistry of lymphomas. *Histochem J* 1983; 15: 655-89.
22. STAVE R, BRANDTZAEG P. Fluorescence staining of gastric mucosa. A study with special reference to parietal cells. *Scand J Gastroenterol* 1977; 12: 885.
23. IWASE T, MORO I, MESTECKY J. Immunohistochemical study of the ontogeny of the secretory immune system. *Adv Exp Med Biol* 1987; 216b: 1359-68.
24. SWTANTARTA S, OGRA PL, LIPPES J, TOMASI JR. TB. Immunohistologic localization of immunoglobulins, secretory component, and lactoferrin in the developing human fetus. *Proc Soc* 1972; 139: 570-4.
25. CONWAY SP, DAAR PRF, SMITH I. Immunoglobulin profile of the newborn baby. *Arch Dis Child* 1985; 60: 208-12.
26. WILSON M. Immunology of the fetus and the newborn: lymphocyte phenotype and function. *Clin Immunol Allerg* 1985; 2: 271-84.
27. DAAR AS, FUGGLE J, FABRE JW, TING A, MORRIS PJ. The detailed distribution of MHC class II antigens in normal human organs. *Transplant* 1984; 38: 293-8.
28. NATALI PG, SEGATTO O, FERRONE S, TOSI R, CORTE G. Differential tissue distribution and ontogeny of DC-1 and HLA-DR antigens. *Immunogenetics* 1984; 19: 109-16.
29. STAGNO S, VOLANAKIS J, REYNOLDS DW, STROUD R, ALFORD CA. Virus host interactions in perinatal acquired cytomegalovirus infections in man. In: COOPER MD, DAYTON DH, eds. *Development of host defence*. New York: Raven Press, 1977.
30. BRANDTZAEG P. Transport model for secretory IgA and secretory IgM. *Clin Exp Immunol* 1981; 44: 221-8.
31. BECKERMAN RC, BUCHINO JJ. Arthrogryposis multiplex congenita as a part of an inherited symptom complex: two case reports and a review of the literature. *Pediatrics* 1978; 61: 417-21.