ABSTRACT

We have reviewed selected aspects of recent findings in the ontogeny of immunity in man. For obvious reasons, constraints placed on experimental work with human subjects, albeit deceased foetuses, limit the work to in vitro studies. Nevertheless, a number of novel and important concepts have emerged. First, as in lower animals, lymphoid development of the foetal thymus in general precedes the development of immunocompetence in peripheral lymphoid tissues. A striking exception to this rule is the finding of cells in early foetal liver which respond to allogeneic cells in the mixed lymphocyte culture some weeks before lymphoid organization of the thymus. In addition, the response of foetal cells to allogeneic cells in the mixed lymphocyte reaction (MLR) precedes the response to phytohaemagglutinin (PHA), a stimulant with relative T cell specificity. The use of markers for T cells to map the emergence of this class of lymphocytes correlates well with various functional attributes of T cells in in vitro culture systems. B cells emerge first in foetal liver at about 9 weeks' gestation, but formation of immunoglobulin by the foetus occurs at very low levels until after birth. There is a suggestion that progression from IgM to IgA synthesis occurs during human foetal lymphoid development. Taken as a whole, these data suggest a rather remarkable and perhaps unexpected degree of cellular and potential humoral immunocompetence at early stages of foetal development in man.
Lymphocytogenesis

Current evidence suggests that primitive mesenchymal cells found in the mesodermal abembryonal pole of the yolk sac wall represent the anlage of the haematopoietic system in man (Weiss 1966; Copenhaver 1964). During the second and third weeks of gestation these stem cells proliferate and differentiate into precursor cells that initiate production of erythrocytic, megakaryocytic, granulocytic, lymphocytic and monocytic cell series. At about the sixth week, haematopoietic stem cells migrate into the developing hepatic parenchyma and their subsequent proliferation makes the liver the major blood-forming organ during early foetal life (Copenhaver 1964). Nucleated red cells are the predominant cells found in the foetal circulation during the second month, but granular leukocytes and megakaryocytes have also formed.

The thymus, the first lymphoid organ to develop, originates at about 6 weeks from the third branchial pouch. The thymus originally is epithelial in nature, but by the eighth to ninth week of gestation, small lymphocytes can be demonstrated histologically within the thymus (Solomon 1971; Papiernick 1970). Direct evidence in man for the origin of these thymic lymphocytes is lacking, but animal studies suggest that they result from migration of hepatic lymphoid precursor cells into the thymic stroma (Owen 1972). Small lymphocytes appear in foetal peripheral blood at seven to eight weeks of gestation. These cells increase in numbers until they comprise over 50% of the leukocytes present in the peripheral blood, and they morphologically are similar to adult small lymphocytes (Solomon 1971). Peripheral blood lymphocytes increase from about 100/µl at 12 weeks to 10 000/µl at 20 to 25 weeks’ gestation.

Subsequent to the thymic lymphoid infiltration, there is considerable increase during the 12th through the 16th week in the lymphoid population of spleen, lymph nodes and bone marrow (Solomon 1971). The spleen appears as an active blood-forming organ early in the third month of gestation; erythropoiesis remains predominant until the fifth month, when red cell production becomes less important and lymphocyte and monocyte production increases (Copenhaver 1964). Haematopoiesis, which can begin in the bone marrow as early as in the first two to three months, normally only assumes importance in the fifth month. With the decline in erythropoietic activity of the liver and spleen in the eighth month, the marrow becomes the major source of erythroblasts and myelocytes (Weiss 1966). The stromal elements of lymph nodes are formed by the third month, but their lymphoid cellular population does not appear until the fourth month. Plasma cells are usually not demonstrable in the lymph nodes of term infants (Silverstein & Lukes 1962).
Concomitant with the rapid increase in haematopoietic cellular differentiation, an increasing number of related serum proteins are being synthesized. Haemolytically active complement is present in foetal cord serum at birth, but in quantitatively reduced amounts (Rosen 1974; Fishel & Pearlman 1961). Using a combination of radioautographic and functional assays, the synthesis of complement components C2 and C4 have been noted in foetal macrophages at eight weeks of gestation (Gitlin & Boesman 1966). C3, C5 and C1 inhibitor proteins are synthesized by hepatic cells, and C1 production by colon columnar epithelium was also demonstrated (Colten et al. 1968). Gitlin and co-workers (Gitlin & Boesman 1966; Gitlin & Biasucci 1969), using radioimmunoelectrophoretic and fluorescent antibody assays, have shown the production of a wide variety of serum proteins by the yolk sac, liver and other tissues. These have included complement components and alpha-foetoprotein at 29 days and ceruloplasmin and orosomucoid at 32 days.

Data on human foetal immunoglobulin production is sparse. Production of immunoglobulin molecules of foetal origin during embryonic life is suggested by the demonstration of paternal Ig allotypic determinants in cord serum from newborns (Martenson & Fudenberg 1965) and by maternal immunization to foetal allotypic determinants in the 7th month of pregnancy (Fudenberg & Fudenberg 1964). van Furth et al. (1965), using foetal spleen cell cultures of infants (13–31 weeks) born alive but who died soon after birth, have documented IgM and IgG synthesis at 20 weeks of gestation. No IgA or IgG synthesis was noted. Gitlin & Biasucci (1969), using a different radioimmunoelectrophoretic assay, have found IgM synthesis at 10.5 weeks, IgG synthesis at 12 weeks, and no IgA synthesis.

Cellular immunity

Thymus-derived cells – T lymphocytes

The formation of rosettes between human lymphocytes and sheep red blood cells (SRBC) is recognized as a property of thymus-derived lymphocytes (Wybran & Fudenberg 1971; Jondal et al. 1972). In fact, investigation of the organ distribution of rosette-forming lymphocytes from human foetuses comprises a major postulate in the notion that the SRBC rosette is a T lymphocyte (Wybran et al. 1972, 1973). In a study of 13 foetuses (11–19 weeks conceptional age) (Wybran et al. 1972), a maximum of 65% rosette forming cells (RFC)
were found in the thymus. However, there was a relatively poor correlation of increasing RFC with increasing foetal age. A small percentage of RFC were found in foetal blood and splenic lymphocytes. In the few bone marrow specimens examined, 0-8% of the cells were positive, probably reflecting contaminating peripheral blood present in this organ. In a modification of the rosette assay in which cells were incubated in foetal calf serum or gamma-globulin-free calf serum, a substantial increase in percentage of RFC in 12 human foetuses was detected (Wybran et al. 1973). As many as 90% of the thymocytes (15 week-old foetus) and significantly higher numbers of splenic and blood lymphocytes formed SRBC rosettes in the presence of serum.

The results of these studies are consistent with the notion that RFC probably originate in the thymus and gradually migrate to peripheral lymphoid organs during embryogenesis. The failure to find that 100% foetal thymocytes form rosettes probably is a function of the particular assays used, but maybe also reflects intrinsic heterogeneity in thymic cell populations at the stages of development investigated. Current studies in this laboratory suggest that 95-100% of foetal thymocytes from 12-20 weeks of gestation react specifically with anti-foetal thymocyte serum by a sensitive immunofluorescence assay (Stites, unpublished observations). In addition, the ontogenic appearance of T cells, both in the thymic and peripheral organs correlates well with less specific functional assays for T cells, e.g., PHA stimulation and MLR (vide infra).

**Bone marrow derived cells – B lymphocytes**

A population of lymphocytes in blood and peripheral lymphoid organs which has easily detectable surface immunoglobulin is bone marrow derived (B-lymphocytes) (Pernis et al. 1971; Cooper et al. 1971; Grey et al. 1971). Lawton et al. (1973) have employed surface Ig markers to map the development of the B cell in the human foetus. The earliest detectable IgM and IgG bearing cells appeared in the foetal liver at 9.5 weeks' gestation. IgA bearing cells first appeared in the liver of an 11.5 week-old foetus. After 11.5 weeks, B cells bearing surface IgG, IgA and IgM were found in liver, spleen, thymus and peripheral blood. A large increase in the percentage of B lymphocytes occurred during 12-13.5 weeks' gestation and by 14.5 weeks, the numbers in peripheral blood approached that found in normal term infants. Although these workers failed to identify the anatomic site at which B lymphocytes first appear (analogous to the avian bursa), their results are consistent with the sequence IgM, IgG and IgA synthesis. Since it has been suggested that a switch from IgM
to IgG occurs in clonal fashion (Kincade et al. 1970; Lawton et al. 1972), it would be interesting to determine if cells staining for surface or cytoplasmic IgG and IgM occur during ontogeny. Employing two different B lymphocyte markers, aggregated Ig for the Fc receptor, and an antiserum specific for chronic lymphocytic leukaemia (B cells), less than 5% positive cells were detected in a series of foetal thymocytes (12–20 week-old foetuses) (Stites, unpublished observations). The number of B cells in cord blood bearing surface Ig has been examined by Fröland & Natvig (1972). The mean value for cells containing F(ab')2 determinants was 14.3% with a range of 5–33%. An average of 9.7% of cells stained for IgM and 7.9% for IgG. Virtually no cells positive for surface IgA were found. Of interest, the dominant IgG subclass of cord lymphocytes was IgG2 and the total number of B cells in cord blood slightly exceeded that in normal adult controls.

Of particular interest is the finding of Rowe et al. (1973) that a disproportionately high percentage of newborn lymphocytes compared to adult controls had surface IgD (14% vs. 3.5%, respectively). This result raises speculation that IgD may be analogous to foetal Ig as haemoglobin F is for the red cell. Further evaluation of the development of B cells in human embryogenesis is needed. Important unresolved questions include: What is the temporal appearance of cells containing Fc receptors, C3 receptors and anti-CLL markers? Is there an orderly progression from IgM to IgG to IgA synthesis as proposed by Cooper et al.? Do B cells precede T cells in ontogeny (or vice versa), and finally, can B cell assays be employed to detect the elusive analogue of the avian bursa of Fabricius in man?

**Antigen binding lymphocytes**

The formation of a bond between lymphocytes and antigen is probably necessary as the initial step in cellular processes which result in subsequent antibody synthesis or cell mediated hypersensitivity. Current evidence suggests, and reasons of genetic economy almost demand, that antigen receptors on lymphocyte membranes be identical to or structurally very similar to immunoglobulin (Warner 1974). Whether cells bearing surface antibody are mainly B lymphocytes, the majority opinion, or whether T cells contain surface Ig, albeit in much smaller amounts, is currently very controversial. If they do, is the surface Ig (probably monomeric IgM) the receptor for antigen? This is also controversial. The emergence of antigen binding cells in the thymus of 22 human foetuses was examined by Dwyer et al. (1972), employing 125IFlagellin and autoradiography. The mean number of antigen binding cells was highest in foetal thymus, 182 per 10⁴ cells, compared to 60 per 10⁴ in children’s thymus or 5 per 10⁴ in the adult organ. Of special interest, binding of radio-
labelled antigen was blocked by preincubation of thymocytes with either anti-\( \mu \) or anti-light chain sera. Although these results do not strictly prove that antigen binding cells were T cells or that surface Ig was the thymocyte antigen receptor, they are suggestive that such was the case. The higher proportion of antigen binding cells present during foetal life in the thymus may be related to the generation of antigen (transplacental) activated lymphocytes in the thymus.

Response of foetal lymphocytes to PHA

Phytohaemagglutinin (PHA) is a plant lectin which has been widely used to evaluate lymphocyte activation in normal as well as diseased humans (Naspitz & Richter 1968). Proliferation of lymphocytes which follows culture with soluble PHA is primarily a function of T cells (Greaves & Janossy 1972), whereas locally concentrated or insolubilized PHA can also stimulate B cell proliferation (Greaves & Bauminger 1972).

Various workers have studied the response of human foetal lymphocytes to PHA. Pegrum et al. (1968), employing a single dose of PHA and autoradiography, detected a four-fold increase in \( ^3 \)H-thymidine uptake in 22 foetal thymic cultures from specimens ranging from 16–24 weeks’ gestation. Foetal splenocytes responded less uniformly and there was little or no response to either bone marrow or liver cell cultures. Kay et al. (1970) were the first to demonstrate response of foetal thymocytes to PHA at 14 weeks’ gestation, and their series contained a number of younger or non-responsive thymocyte preparations. A correlation existed between the onset of thymic PHA responsiveness, the demarcation of thymus into cortex and medulla and the development of a rise in peripheral blood lymphocyte counts (Playfair et al. 1963). Papiernick (1970) correlated the response of PHA stimulation in the 22 thymocyte specimens from foetuses aged 12–22 weeks with morphologic changes. Until the 18th week of gestation there is a progressive increase in degree of transformation. Thereafter, the level declined to that of adult thymic lymphocytes. She concluded from morphologic study that the thymic medulla was the probable source of PHA responsive cells. August et al. (1971) confirmed and extended these findings in a study of 18 human foetuses from 12–28 weeks’ gestation. He found the appearance of PHA responsive cells in foetal spleen (14–16 weeks’ gestation) approximately 2 weeks following the appearance of this cell population in the thymus.

All of the foregoing studies were done employing a single dose of PHA and harvesting cells for analysis of DNA synthesis at a single time period. Stites et al. (1974) measured time-dose kinetics of the response of lymphocytes from 22 foetuses (5–19 weeks) to PHA. PHA responsiveness was first noted
in thymus at 10 weeks and 3–4 weeks later in peripheral blood and spleen. Marrow and hepatic lymphoid cells failed to respond to the mitogen. A tendency for increased PHA induced DNA synthesis with increasing foetal age in all responsive organs was also observed. Careful dose-response kinetics, especially when studying thymocytes, is of paramount importance. The foregoing studies clearly document the rather remarkable and early appearance of at least one functional attribute of foetal thymocytes and peripheral lymphocytes during development.

Response of foetal lymphocytes to allogeneic cells

Employing the unidirectional mixed lymphocyte reaction (MLR), lymphocyte defined (LD) histocompatibility differences may be detected in non-immunized allogeneic subjects. Whether the responsive cell in the MLR is a B or T lymphocyte, or both, is controversial at present, but evidence has been presented which suggests that PHA and allogeneic cells stimulate different cell populations (Colley et al. 1970; Folch & Waksman 1972; Carr et al. 1973). The MLR has been used both to assess the immunocompetence of foetal lymphocytes and to estimate the presence of LD antigens at early stages of development (August et al. 1971; Stites et al. 1974; Pegrum 1971; Meo et al. 1973). Pegrum first reported that foetal lymphocytes from thymus, liver and occasionally spleen could respond both to normal adult and leukaemic lymphocytes in vitro. August et al. (1971) showed MLR responses in two foetal thymocyte specimens aged 15 and 16.5 weeks of gestation. Carr et al. (1973) emphasized a marked dichotomy between the strong response of foetal liver lymphoid cells to allogeneic lymphocytes and their failure to respond to PHA. In the same study, they showed strongly reactive cells in MLR with foetal spleen and thymus as well. The fact that MLR responsive and PHA non-responsive cells were detected at 10 weeks' gestation suggested that MLR response is congenitally a more primitive response than PHA responsiveness. Similar results have been reported by Meo et al. (1973).

In a detailed study of the time dose response kinetics of MLR responsive cells in 22 human foetuses ranging from 5–20 weeks' gestation, Stites et al. (1974) reported finding MLR responsive cells in foetal liver as early as 7.5 weeks' gestational age. This was the earliest cellular immune reaction detectable in the foetus and clearly preceded lymphoid development of the thymus which occurs between the 10–12 weeks of gestation. The identity of the MLR responsive foetal hepatic cells remains obscure. Lafferty et al. (1972) have suggested that allogeneic cell interactions represent a unique form of immune reaction, distinct from usual antigenic activation. By analogy with their work in chicken, the liver MLR response in human foetuses could result from stem
cell proliferation. No direct evidence for this postulate exists, however. A unique primordial role for hepatic cells in establishing immunocompetence in the mouse was demonstrated by Tyan (1968). Giller et al. (1972) have produced allogeneic radiation chimeras in mice across strong H-2 barriers by injection of foetal liver cells and small numbers of thymic lymphocytes. The finding of a few patients with severe combined immunodeficiency diseases (SCID) in whom PHA non-reactive MLR reactive cells are present in peripheral blood suggests that a developmental defect in T cells, but not so-called “hepatic dependent” lymphoid cells, exist (Seligman, personal communication; Stites et al., unpublished observations; Meuwissen et al. 1968). Recently, Cooper et al. (1971) have reported initial success in immune reconstitution of a patient with SCID with an allogeneic foetal hepatic graft. Certainly the use of this fertile source of stem cells and immunocompetent lymphoid precursors warrants further trial in patients without histocompatible sibling donors.

**Effector cells activity in human foetal lymphocyte populations**

In addition to causing proliferation of susceptible lymphocytes, PHA can also induce non-specific target cell destruction of various cells in vitro (Holm & Perlman 1967). Cytotoxicity thus produced is non-specific since it does not require prior sensitization as does antigen specific target cell destruction. Employing chicken red blood cells labelled with $^{51}$Cr and the release of this radioisotope as an index of lymphocyte mediated cytotoxicity, Stites et al. (1972) examined the responses of a number of lymphoid tissues from foetuses ranging from 14–18 weeks’ gestational age. Whereas thymocytes reacted to PHA with marked proliferation, they failed to produce target cell destruction. Precisely the opposite pattern of in vitro responses was noted with foetal bone marrow specimens. The peripheral blood and splenic lymphocytes from the same foetuses responded to PHA with proliferation and xenogeneic target cell destruction. Liver lymphoid cells failed to respond in either test. These results further emphasize the marked segregation of in vitro cellular immune functions during embryogenesis and suggest that functional anomalies in the development of these various cell populations could be reflected in certain genetic immunodeficiency disorders.

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REFERENCES


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DISCUSSION

Nossal: Dr. Fudenberg has given us the opportunity to address ourselves once again to the so-called K cell. I think it is important for the non-immunologists here to realise that of the various cytotoxic mechanisms for grafts, for tumours and presumably also for foetuses, that what we have as analytic tools in the laboratory, there is a great deal of confusion as to which of these really has the greatest in vivo relevance. For example, I think there is a swing towards the view that the blocking factors and cytotoxic tests so beautifully studied and promoted by the Hellström's (1969) may not be as usefully predictive of cancer patients' immediate future as was initially hoped. I think the technology for purifying K cells and hopefully for seeing how good they are in “real life” situations is now at hand. Very recently, Dr. C. R. Parish from Australia, in work as yet unpublished, has worked out a nice methodology for separating these cells from the bulk of mouse spleen cells. It turns out that K cells are negative, as Dr. Fudenberg said, for both immunoglobulin on the surface (a B cell marker) and for the theta antigen which marks T cells. They are positive for the Fc receptor. In fact, they do their job by trapping antibodies passively onto their surface, which allows them to be closely applied to a target cell. So, the antibody attaches to the K cell by the Fc receptor, forming a bridge to the target. It turns out, interestingly enough, that these K cells are non-phagocytic. What Parish has done is to remove from the population all Ig positive cells by a rosetting procedure, all theta positive cells, all phagocytic cells by exposing the population to iron filings and using a magnet. He is left with a very small subpopulation of cells which appears to contain all of the K cell activity of the whole spleen, and these can be rosetted out as Fc positive. So K cells seem to be Fc positive, complement receptor positive, Ig negative, theta negative and non-phagocytic.

My last remark relates to Dr. Fudenberg's apparent concern about the presence of autoantibodies in normal people, as if this were some kind of refutation of Burnett's ideas of self-tolerance. Had Dr. Fudenberg listened carefully to my remarks about the nature of antibody yesterday, he would have realised that it is really quite impossible that we should not have low affinity antibodies to ourselves in our own bloodstream. This is getting back to the point that I made, that the generator of diversity faced
with this enormous task of recognizing any antigen in the universe, works in a very
degenerate kind of way. Antibodies against S. typhi, plague and smallpox and so forth
may well have low affinity crossreactivity to self-antigens. This is in fact dependent
on the sensitivity of the antibody detection method. I dare say autoantibodies of low
affinity are not doing us any harm at all.

Fudenberg: First about K cells. Two weeks ago we had a WHO sponsored meeting
in London on human T and B cells; most laboratories had exactly the same results.
It appears that human peripheral blood mononuclear cells are negative for surface 19,
negative in F-rosette red, positive in the E-AC rosette test and which bind IgG segre-
gates, these cells include the K cells complement positive, not phagocytosis. These cells
are not phagocytic by the usual criteria, but this does not mean that there are not
some kind of monocytic cells or precursors thereof.

In terms of the other point I totally agree with Dr. Nossal. I merely wish to point
out that in Burnett's original book on the clonal selection theory (Burnett 1959) he
said that autoantibodies are due to emergence of forbidden clones and all normal
people, but not agammaglobulins, have autoantibodies of several kinds.

Miller: You mentioned that IgM and IgG-bearing cells first appeared in foetal liver
at about 9-10 weeks of gestation. Have you or anyone else looked at IgD-bearing cells
to determine when they first appear?

Fudenberg: We have not done that. I think Dr. Pernis may have; he and Dr. Rowe
at Lausanne were the people most likely to do that.

Tatwar: Dr. Fudenberg, I would like to turn to your remarks about the alpha-foeto-
proteins as a possible blocking factor in pregnancy sera. There are certainly many
components which increase during pregnancy and many of these, when tested in
in vitro systems, give inhibitions. Some of the pregnancy hormones which increase
during pregnancy, when tested in vitro, would depress the transformation with PHA.
This does not necessarily mean that they are blocking factors in vivo. Do you have
any further critical evidence to believe that alpha-foetoproteins play this role?

Goodman: At a recent meeting, in March in Nice, on alpha-foetoprotein, my under-
standing was that the early promise by Caldwell et al. (1973) and Parmely et al. (1973)
was not borne out. In the discussions, Belanger (1974) stated that alpha-foetoprotein
was cytotoxic to lymphocytes, and this might account for the finding of in vitro sup-
pression of lymphocyte reactivity. In addition, he found no failure of lymphocyte
reactivity in patients with tyrosinaemia, nor in rats who have elevated serum alpha-
foetoprotein levels.

David: Concerning the presence of several subpopulations of lymphocytes, I would like
to relate some recent findings of MacDermott, Chess and Schlossman who have been
studying the function of various populations obtained by purification on immuno-
absorbent columns. They examined the populations which were not absorbed to the
anti-Fab column (cells with no immunoglobulin on their surface) and removed the
T cells by making E rosettes. The remaining cells which did not form T rosettes
(about 15% of the non-immunoglobulin-bearing lymphocytes) had receptors for EAC.
So far, these cells appear to function in a manner indistinguishable to B cells, except
that they do not have immunoglobulin on their surface, nor produce Ig in culture.
These cells can mediate antibody induced cytotoxicity.
Fudenberg: The consensus of the recent I. V. I. S.-WHO – B & T cell reacting in dose meeting was first of all that one must have to take the mononuclear cells off Ficoll-Hypaque gradients by a standard method. How far one puts the pipette into or below the cell layer makes a big difference. Further, if one does not stain for IgD, one gets only 15% of cells positive for Ig markers. If one does stain for IgD, one is left with – in most laboratories – 2–4% of surface immunoglobulin negative, rosette negative cells; these possess Fc receptors and often possess complement receptors but lack the two major markers, namely Surface Ig and E-rosette formation.

The other finding is that in some types of immune deficiency the number of these Ig negative, E-rosette regulated cells is vastly increased. One finds up to 15–20% of such cells in certain patients with immune deficiency, maybe as a compensatory phenomenon, I do not know. I would not know how to interpret this without knowing how Dr. David’s group defined their K cell.

Talwar: Is alpha-foetoprotein specific to human or is it crossreactive to other animals?

Vaerman: I know of a paper by some Japanese people (Nishi et al. 1972) showing clearly crossreactions between alpha-foetoproteins of at least 5 or 6 species, including the human.

Simons: I can confirm that Hirai, Nishi, Watabe and Tsukada in Japan have demonstrated crossreactivity between antisera prepared against purified human AFP in at least 8 or 9 species (Hirai et al. 1973).

Goodman: The same investigators, and also Abelev and his group in Moscow (Abelev 1973) have shown that by injecting heterologous alpha-foetoprotein one can produce autoantibodies in rats to rat alpha-foetoprotein, so perhaps there you have an approach to a contraceptive method.

References: