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Development of Immune Mechanisms*

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The development of the immune response may be considered as a series of adaptive cellular responses to a changing and potentially hostile environment. It may be considered at several levels: the species, the individual or the cell.

The effect of the hostile environment ensures survival by selective pressures of those life forms within the species which were best adapted to that environment. This adaptive process forms the basis for the *phylogeny* of the immune response. The microenvironment in which undifferentiated immunologic progenitor cells exist, provides yet another type of inducing stimulus with the developing individual *(ontogeny)*[1-3] The immunologically mature individual may be considered as the selected form which resulted from this type of development. Finally, when cells find themselves within the molecular milieu of antigen, a series of proliferative and differentiative events occur characteristic of the immune response. This leads to the synthesis of cell products such as antibody or mediators of delayed hypersenstitivity.

The most primitive manifestation of a resistance mechanism is phagocytosis. It is found in the most ancient of the unicellular organisms, and served a nutritive function: In higher forms, the process evolved to a defense function. From the presently identifiable life forms, there arose an increasing complexity of the immune system, ranging from the primitive defense of phagocytosis to the humoral cell-mediated responses characteristic of specific immunity. As cellular life differentiated into more complex forms, there developed an increasing specializa-

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tion of the systems concerned with recognition of foreigness. For instance, a newly acquired mesodermal layer is added to defense function. In the higher invertebrates, a vascular system developed which allowed phagocytosis to proceed both by fixed and circulating cells. Thus in phylogeny the two most important nonspecific elements, phagocytosis and the inflammatory response were formed in primitive life forms. With evolution, specific immunity and biologic amplification systems are added The latter comprise in the primary system the following: opsonins of γG and γM types, γE release vasoactive substances, cell mediated low molecular weight effector molecules. In the secondary system kinins, clotting system and the complement which augment inflammatory response are to be mentioned. Here the older mechanisms were not replaced with evolution, but rather were continued and reinforced as evolving life-forms added new responses.

Phylogeny of Specific Immunity

The first evidence of a specific immunologic system appeared in primitive vertebrates, such as the hagfish.[3] In these early species there existed a primitive high molecular weight antibody and cells which could manifest cell mediated immunity. In the elasmobranches, for example, a tissue transplant consisting of skin or scales, would be promptly rejected. Introduction of antigens into these species will elicit a high molecular weight antibody analogous to γM immunoglobulin of higher forms. During evolution there occured specialization of cells and supporting structures to house these tissues—the lymphoidal organ to appear in phylogeny and is present in the most primitive of vertebrates. There also appeared in higher forms, the birds, a separate anatomical structure arising from the primitive gut, the bursa of fabricius, important in the development of cells which elaborate antibody in avian species. The mammalian equivalent of this organ is not known.

In the most primitive vertebrates, the predominant antibody is a high molecular weight substance, analogous to the γM globulins of the human. During phylogeny, a second class of antibody is elaborated with properties similar to the γG globulins. The γA immunoglobulins appear as rather evolutionary events and are restricted to mammals. The development of a novel form of γA immunoglobulin, the secretory γA globulins, was also elucidated in mammals. This external antibody system has proved to be of immense importance in defense at body surfaces and appears to be a mechanism by which lower forms such as the ungulates, receive passive antibody via colostrum. The major transfer of antibody in the human occurs primarily via the placenta. However,

breast milk still continues to be a source of γA antibody in the human. Finally still later additions seen in man are the γD and γE immunoglobulins. The precise function of γD globulins is unknown, the γE immunoglobulins seen also in other mammalian forms, are a unique class of antibodies involved in immediate-type hypersensitivity.

Genetic Control of The Immune Response in the Developing Individual: Ontogeny

The maturation of the immune response in the human begins in utero sometime during the second to third month of gestation. The differentiation of cells destined to perform both non-specific and specific immunologic functions, appear to have a common ancestral origin. Both cell types appear to arise from a population of progenitor cells referred to as stem cell or hemocytoblasts; these are located within the hematopoietic tissues of the developing embryo (yolk sac, fetal liver and bone marrow). Depending upon the microchemical environment surrounding the cells, development will occur along at least two avenues: the hematopoietic and the lypmphopoietic.[4] The former lead to proliferation and differentiation to blood forming precursor cells. The latter forms the lymphoid precursor cells which can differentiate along two additional pathways. The first, under the influence of the thymus, perhaps within the substance of the gland itself, includes a population of small lymphocytes which subserve the function of cell mediated immunity (thymic dependent or T lymphocytes). These functions include the delayed hypersensitivity (tuberculin type) reactions, the ability to recognize and to reject solid tissue homografts, defense against viral, fungal and bacterial infections, immunity against tumors, blastic transformation and g.v.h. reaction. Recent evidence suggests that a thymic hormone may be operative in this expansion. This population of lymphocytes circulates throughout the lymphatic and vascular systems (recirculating pool) and populates lymph nodes in thymic dependent regions. In thymectomized animals, this zone is depleted. Such animals display a profound reduction in circulating and tissue lymphocytes and show impaired cell-mediated immune functions. They are unable to manifest delayed hypersensitivity and show prolonged graft survival with little or no effect on their antibody functions.

If the progenitor cells come under a second type of microchemical environment, differentiation will occur to produce a population of lymphocytes and plasma cells concerned with humoral immunity or antibody synthesis. This population of cells comes under the influence of and is expanded within a second anatomic site, whose identity as the bursa of Fabricius is known with certainty only in birds. This population

of cells is also part of the circulating pool of lymphocytes and populates the thymic independent regions of lymphoidal tissue. Removal of the bursa or its mammalian equivalent, leads to a profound deficiency of gamma globulin with little or no effect on cell-mediated immunity, A depletion of the thymic independent regions of lymphoid tissue is also observed.

The functional significance of this two-compartment system is important to clinical medicine and according to the involvement of thymic dependent, or independent, or both systems various immunologic deficiencies develop.

After reviewing the development of systems for cellular and humoral immunity, we would now like to take a quick look at the immunity in the fetus and the newborn in general.

Transfer of Immunoglobulins and Specific Antibodies[5-12]

Transfer of immunoglobulins from mother to offsprings occurs in many species. In the chicken, transmission takes place from the hen to the ovum via the follicular epithelium and then to the embryo via the yolk sac. In mice, rats, rabbits and guinea pigs it occurs by way of the fetal yolk sac, not by the placenta, and it also takes place by means of colostrum and milk. In pigs, calves and lambs, there is practically no transfer of immunoglobulins and since none is produced by the fetus, the young are born essentially agammaglobulinemic. They receive maternal immunoglobulins only after birth through the colostrum and milk and thus via the intestinal epithelium. Table I.

TABLE I

ROUTES OF PASSIVE TRANSFER OF IMMUNOGLOBULINS FROM MOTHER TO FETUS

Species	Route of Transfer: Yolk sac	Placenta	Colostrum or milk	References
Avian	+			Paterson et al. 1962[5] Weller and Schecthman 1962[6]
Rodents	+	—	+	Brambell 1970[7]
Porcine	+	∓	+	Mason, et al. 1930,[8] Brambell 1958[9]
Ovine	—	—	+	Brambell 1958[9]
Primates	—	+	—	Bangham 1960,[10] 1961,[11] Schneegans[12] et al. 1962.[12]

There are different pathways of transmission of maternal antibody to the fetus in different species (Table II).

In species with large numbers of membranes intervening between the maternal and fetal circulations, the colostral route seems to be a more important mechanism of transfer. Conversely, as the number of layers decreases, as in man, the placental route seems to have assumed greater importance (Table II).

TABLE II

RELATIONSHIP OF TYPE OF PLACENTATION WITH CHARACTER OF MATERNAL FETAL TRANSFER OF ANTIBODY IN VARIOUS SPECIES[13]

Animal	Number of Placental Membranes	Relative importance of route	
		Placental	Colostral
horse	6	0	+++
sheep, cow	5	0	+++
cat, dog	5	+	++
rat mouse	4	+	++
rabbit, guinea pig	3	+++	∓
man, monkey	3	+++	0

In the primates, including man, a high concentration of immunoglobulins exists in milk, but the intestinal route does not constitute an important mode of transfer. Instead, immunoglobulins are transferred during gestation across the placenta. Only the γG component is transmitted, by virtue of a receptor located on the Fc fragment of the molecule. No detectable amounts of γM or γA appear in the fetus. Passive transfer of specific antibodies in man occurs at about the same time as that of immunoglobulins but only antibodies belonging to the γG class are transferred. No γA or γM globulins are present in cord sera. This is due to the fact that the fetus is usually protected in utero from antigenic stimuli.

The question of passive transfer of maternal immunoglobulins is important for two reasons:

1. It provides protection to the offspring before it can develop its own efficient immunological mechanisms.

2. Passively transferred antibody interferes to some extent with the active synthesis of that antibody by the infant.

Response to Antigenic Stimulus

In normal circumstances the body will respond immunologically only to antigens foreign to it and not apart from a few exceptions, to native substances.

a. Immunological Tolerance

In 1945 Owen described the existence of erythrocyte mosaicism in dizygotic cattle twins and attributed it to an interchange of hematopoietic stem cells through vascular anastomoses between the twins.[14] Burnet and Fenner in 1949 postulated that at some stage in embryogenesis the fetus developed a recognition mechanism by which all antigens present during a critical period were acknowledged as self antigens and unable to stimulate henceforth an immune response.[15] Antigens encountered after this period could be recognized as foreign and capable of evoking a state of immunity. It was originally thought that tolerance could be induced only in immunologically immature animals. It is now known, however, that chronological age is not the all important factor in determining whether an antigen will induce tolerance or immunity. Factors such as the type and relative dose of antigen, the efficiency of the reticuloendothelial system in adequately processing the antigen, and the existence of immunologically competent cells at certain sites in the host, appear to determine the outcome. Thus tolerance can be induced in immunologically mature animals even by low dosage methods. Conversely, immunity can be induced in most neonatal animals by giving appropriate doses of antigen. Furthermore a state of tolerance, once induced, will be lost as a new population of virgin immunocompetent cells is produced and when antigen is no longer made available to paralyse these new cells.

b. Immunoglobulin Synthesis.

It has generally been found that little or no synthesis of any of the immunoglobulins normally occurs in the developing fetus. Thus, when there is no transfer of maternal immunoglobulins to the fetus as in the porcine, bovine and ovine species, the young are born practically agammaglobulinemic. When there is transfer, as in the primates, the serum γG globulin level of the newborn is as high as that of the mother. In man, after birth the level begins to fall, reaching a plateau (100 mg/100ml serum) at 3-10 weeks and then rises again to reach adult levels (600-1500 mg/100 ml) between 1 and 4 years of age.[16] Normal children born of agammaglobulinemic mothers were agammaglobulinemic at birth (10 mg/100 ml serum) and exhibited low γG levels for 1-2 months, after which the concentration rose to reach the normal range by six months of age. Some normal children show a delay in the production of normal amounts of γG, a condition termed transient agammaglobulinemia. Children who are born of mothers with normal γ globulin levels, but who are destined to become agammaglobulinemic, had normal levels

of γ globulin at birth, these declining to remain at the low concentration of 20 mg/100 ml or less[17]. The postnatal rise of immunoglobulin levels, occurs as a result of exposure to antigens present in extra uterine environment. In healthy children the γM levels begin to rise during the second and third months of life. The γM globulins attain adult levels by one year of age, the γG globulins by five to six years of age and the γA globulins by 10 years of age.

Studies of Gitlin and Biasucci in 15 normal human embryos and fetuses of 29 days to 18 week gestation showed that the synthesis of plasma proteins begin in the early days of gestation.[18] The human embryo as early as 29 day gestation synthesized β_1C/β_1A, C_1 esterase inhibitor, transferrin, hemopexin, α_1 antitrypsin, β lipoprotein, α_2 macroglobulin and prealbumin.[18] At 32 days gestation, ceruloplasmin and orosomucoid were also synthesized, but synthesis of fibrinogen was not observed before 5.5 weeks. Synthesis of γM occured as early as 10.5 week gestation and γG synthesis was found in cultures as early as 12 week gestation. γA synthesis was not detected in any of the tissue cultures. With the exception of gamma globulins, each of the proteins studied was synthesized by the liver, but additional sites of synthesis for some of these proteins were also found. Synthesis of γG and -γM occured primarily in the spleen, but other sites of synthesis were noted as well.

Van Furth and colleagues studied 20 fetuses aged between 13 and 31 weeks.[19] The results of the spleen cultures demonstrated the synthesis of IgG and IgM which starts at about the twentieth week of gestation. In the serum, IgM could be detected at about the same period. The immunofluorescent staining of the spleen tissue showed that medium sized and large lymphoid cells as well as plasma cells even with Russel bodies were positive for either IgG or IgM. The peripheral blood was also found to contain a small number of medium sized IgG and IgM positive cells. Both the spleen and the peripheral blood showed a considerable number of fluorescent small lymphocytes which exclusively contained IgM. The relatively high ratio of IgM to IgG production prenatally as compared to the postnatal situation, agrees with a predominantly primary antibody response in fetal life. No indications for the synthesis of IgA and IgD during fetal life were found. From these studies one can say that during the second trimester of gestation the human fetus reaches a situation comparable to the one found in adult lamprey; this animal being the most primitive of vertebrates to show the presence of immunoglobulins, antibody production and proliferation of lymphoid cells after antigenic stimulation, lymphoid cells in the peripheral blood and small lymphoid foci in the spleen.

Recently in the human fetus, B cells bearing IgM, IgG and IgA surface determinants have been demonstrated in peripheral blood, bone marrow, liver and spleen at 11.5 weeks of gestation by Lawton and coworkers.[20] By 14 weeks the percentage of cells in the circulation having each class of receptors is equivalent to that found in adults. Cells containing cytoplasmic immunoglobulin were not found at either age.[20]

Fudenberg and Fudenberg demonstrated the synthesis of IgG in the human fetus at the seventh month of gestation by the presence of anti-Gm (a) agglutinins in the serum of the mother in a case of maternal-fetal incompatibility for Gm (a),[21] Using the same approach, this finding was extended by establishing the presence of IgG in the cord blood synthesized by human fetus.[22]

c. Production of Specific Antibodies.

It used to be considered that most mammals matured immunologically only after birth and immunological tolerance could be induced most easily at birth in many animals and that the newborn of many species responded poorly, if at all, to a varient of antigenic stimuli. Thus for instance, the responses of infants under one month of age to some vaccines were poor.[23] Furthermore, the newborn of many species has a very immature lymphoid system devoid of antibody-producing plasma cells. This, however, appears to reflect on the capacity of the normal placenta to shield the offspring from exogenous stimuli rather than on the incapacity of the fetus to produce plasma cells. The human fetus can actively produce Wassermann antibodies and both congenital syphilis and toxoplasmosis are associated with formation of plasma cells in fetuses as young as six months gestation.[24] Premature infants can gain full immunological competence at the same rate as normal full-term infants.[25] Animals raised under sterile conditions after birth, on the other hand, have immature lymphoid tissues and a rarity of plasma cells.[26]

It seems, therefore, that immunological maturation is not coupled to the birth process, and that an environmental factor, presumably antigenic stimulation, is responsible for the expression of immunological capacity.

With recent studies it has been shown that the capacity to produce humoral antibodies to some, but by no means to all antigens, is present in many species at birth.[27-36] (Table III) and even in fetal life (Table IV).[37-41] Although antibodies can be detected, their rate of production and the maximum amount produced is reduced in comparison with older animals. Adult capacity in rodents may not be achieved until some weeks after birth.

TABLE III

ANTIBODY PRODUCTION BY THE OFFSPRING OF VARIOUS SPECIES CHALLENGED ON THE DAY OF BIRTH

Species	Antigen	Evidence of Immunization	Age When Antibody Was Detected (Days)	References
Mouse	Pneumococcal Polysaccharide Type II.	+		Siskind, Paterson and Thomas (1963)[27]
	Bovine γ-Globulin	+		Thorbecke, Siskind and Goldberger (1961)[28]
Rat	Brucella Abortus	+	9–10	Halliday (1964)[29]
Guinea-Pig	BCG	+	15	Bishop and Gump (1961)[30]
	Bovine γ-Globulin	+	15	
	Brucella Abortus	+	15	
Rabbit	Salmonella (Flagella) (Large Doses)	+	10	Sterzl and Trnka (1957)[31]
	Foreign Erythrocytes	+	5	Bellanti, Eitzman, Robbins and Smith (1963)[32]
	Human Serum Albumin	—		Riha (1962)[33]
	Salmonella (Flagella)	+	7	Riha (1962)[33]
Pig	Tetanus and Diphtheria Toxoids	+	14	Segre and Kaeberle (1962)[34]
	MSP-8 Bacteriophage	+	3-4	Kim, Bradley and Watson (1966)[35]
Man	Salmonella (Flagella)	+	7	Smith and Eitzman (1964)[36]
	Salmonella (Somatic)	—		

TABLE IV

FIRST EVIDENCE OF IMMUNE RESPONSE IN ANIMALS CHALLENGED IN FETAL LIFE

Species	Gestation Period	First Appearance of Small Lymphocytes	Antigen	Earliest Appearance of Antibody or Earliest Capacity For Skin-Graft Rejection	References
Frog	1 Year (Larval)	40-45 Days After Hatching	Gold-Fish Serum Protein	60± Days After Hatching	Cooper and Hildemann (1965)[37]
		Skin Homograft			
Opossum	12.5 Days (Uterus)	7-11 Days After Birth	Φ X 174	11 Days After Birth	Kalmutz (1962)[38]
	60∓ Days (Pouch)		Salmonella Typhi	8 Days After Birth	Rowlands, Lavia And Block (1964)[39]
Lamb	150 Days	35-40 Days' Gestation	Φ X 174	41 Days' Gestation	Silverstein, Uhr, Kraner and Lukes (1963)[40]
			Ferritin	66 Days' Gestation	
			Skin Homograft	85 Days' Gestation	
			Ovalbumin	125 Days' Gestation	
			Salmonella Typhi	42 Days' After Birth	
			Diptheria Toxoid	42 Days' After Birth	
			BCG	42 Days' After Birth	
Calf	280 Days	60 Days' Gestation	Leptospira Saxkoebing	132 Days' Gestation	Fennestad and Borg-Petersen (1961)[41]

The achievement of immunological competence may not be effected toward all antigens at one and the same time: an antibody response to one antigen can be detected in the offspring before a response to another. For instance, the larvae of the poikilometric vertebrate bull frog can produce antibodies to serum isoantigens, gold-fish serum proteins, crayfish serum, bovine serum albumin and bacteriophage T7, but not to influenza A strain PR-8 when challenged between 60 and 200 days of larval life.[37] A fetal lamb near term can respond to bacteriophage Φ X 174, ferritin and ovalbumin, but not to diphteria toxoid, Salmonella typhi flagella and BCG[40] (Table IV). Whether this hierarchy in the response reflects on the strength of the antigenic stimulus, the sensitivity of the antibody-detection technique, the varying capacities of different antigens to penetrate cells or be phagocytosed by cells at a certain stage of development, or on a true maturation of different immunological specificities at different times, is not clear.

The first antibodies to appear in the circulation in fetal lambs, human infants and other species belong to the γM class. From 30 to 40 days later γG globulin appears. In the adult, the first antibody to appear after primary immunization is also of the γM class, but it is followed in 3-4 days by the appearance of γG. Qualitatively, therefore. the pattern of primary immunization in the adult and newborn is similar. Only the timing of appearance of the various classes of antibodies is different.

d. Delayed Hypersensitivity.

Tuberculin sensitivity resulting from BCG vaccination at birth in human infants appeared between 2 and 3 weeks after inoculation, as in adults.[42] Premature and fullterm newborn infants, however, were not as readily and as regularly sensitized by the percutaneous application of 2,4 dinitrofluorobenze as older infants.[25] About one third of guinea pigs injected 1-2 weeks before birth with diphteria toxoid, ovalbumin or bovine serum albumin in Freund's adjuvant developed tuberculin type hypersensitivity when a skin test was made on the day of birth.[25,43] These and other studies suggest that the capacity to develop delayed sensitivity appears in some species during late embryonic life. However, the maturation to adult capacity may not be completed until some weeks or months after birth.

e. Homograft Rejection.

Skin homografts applied to the fetal lamb before 75 days gestation are completely tolerated but at 85 days they are rejected within 7-10

days as vigorously as is the case with adult sheep.[44] The rejection by the fetus is performed in the absence of plasma cells and circulating immunoglobulins. Furthermore, injection of anti-sheep γG or γM had no effect on the rejection process, thus suggesting that the presence of circulating antibody is not necessary for first-set graft rejection.[45]

The offspring of other species are also capable at the time of birth or hatching, of rejecting homografts whether in the form of solid tissue grafts or dispersed foreign cell: frog, chick, duck, turkey, mouse, rat rabbit, pig and man. In some species such as the rabbit, lamp, pig and probably man, the capacity for homograft rejection has, or appears to have, developed before birth. In other species such as the bird, mouse and rat, full adult capacity is not reached until some time after birth or hatching. In fetal monkey typical histological reaction of skin homografts is observed as early as day 58 of gestation (fullterm is about 165 days).[46, 47]

Development of Lymphoid Tissues and Their Role in Immunogenesis

The faculty of immunological response is known to be a function of the lymphoid tissues. Direct evidence has been obtained to show that the small lymphocyte is an immunologically competent cell and can initiate some types of immunological reactions. Indeed no immune responses can be evoked from animals before small lymphocytes have appeared at least in the circulation. In all the vertebrate species so far studied, the thymus is the organ in which lymphocytes can be clearly identified and it is closely followed by the tonsil in mammals and the bursa of Fabricius of birds (Table V). Other tissues (lymph nodes, spleen, Peyer's patches, lymphoid aggregates along the intestinal tract) become lymphoidal later in fetal life or even after birth. In marked contrast to the thymus and bursa, their state of development remains primitive until well after birth and they fail to undergo further development if the animals are kept in the germ-free state. Furthermore, plasma cells and germinal centers which are not normally present in the normal animal, presumably as a result of exposure to the extra uterine environment that is not germ-free.

Experimental work in recent years has shown that the thymus and bursa plays a crucial role in the normal development of cells that will subsequently be called upon to participate in immune reactions. Thymectomy in bull-frog larvae before 30 days of age and in neonatal chicks, mice, rats and hamsters strongly interferes with the capacity of the mature animal to reject foreign skin homografts and develop delayed hypersensi-

TABLE V

LYMPHOID DEVELOPMENT IN VARIOUS SPECIES—TIME INTERVAL (DAYS) FROM CONCEPTION TO FIRST APPEARANCE IN LYMPHOID TISSUE

Species	Gestation Period (Weeks)	Thymus (Weeks)	Bursa of Fabricius	Spleen (Weeks)	Lymph. Nodes (Weeks)	Lymp. Tissue Assoc. with Intest. Tract (Weeks)	References
Chick	21	10–12	14	> 20	–	> 18	Ackerman and Knouff (1964)[48]
Opossum	12 ½	13	–	37	16	40	Block (1964)[49]
Mouse	20	14	–	> 20	> 20	> 20	Miller and Osoba[50]
Rabbit	32	20	–	28	36	40	Archer, Papermaster And Good (1964)[51]
Dog	60	30 ±	–	54	48	60 ±	Kelly (1963)[52]
Man	40	12	–	20	20	25	Kay, et al. (1962)[53]

tivity. In some species, notably the amphibians and rodents, it is also associated with some dimunition in the capacity to produce a normal antibody response to some but not to all antigens. In chicks, thymectomy has no effect on serum antibody production, but bursectomy at birth or prenatal inhibition of bursal development by administration of testosterone during incubation prevents antibody and globulin synthesis. In the rabbit and in other species in which the lymphoid development at birth is more advanced than is the case with chicks and rodents, thymectomy has little or no effect on the capacity to perform immune reactions in early life.

It is evident that the thymus and bursa differ from other lymphoid organs in their origin and structure, in their reaction to antigenic stimuli and in the effect of their removal (Table VI). The anatomical and functional defects occuring in lymphoid tissues following the early remo-

TABLE VI

DIFFERENCES BETWEEN PRIMARY AND SECONDARY LYMPHOID ORGANS

	Primary Lymphoid Organs	Secondary Lymphoid Organs
Organs	Thymus (All Vertebrates) Bursa of Fabricius (Birds) ? Tonsil (Mammals)	Lymph Nodes, Spleen, Lymph Tissue associated with Intestinal Tract
Origin of Anlage	Ectodermal-Endodermal junction	Mesoderm
Lymphoid Development	Early in Embryonic (or Larval) Life	Late in Fetal Life or after Birth
Persistence	Involute in adult life	Persist throughout life
Lymphoid Cell Mitotic Activity	High (0.7-1 % In Thymus); Independent of Antigenic Stimulation	Low (0.05 - 0.1 %) Lower In Germ-Free Environment
Plasmacytopoiestis Germinal Centre Formation	Insignificant on nonexistent-even after Antigenic challenge	Characteristically Occurs after Antigenic Stimulation
Effect of early Removal	i. Fall in Lymphocyte Population ii. Early defects in Immune Capacity	Insignificant
Effect of Late Removal	i. Fall in Lymphocyte Population ii. Late defects in immune capacity (after months or years)	Inconsistent and usually not severe

val of the thymus and bursa have suggested that there are primary lymphoid organs that control the development of cells found in the other lymphoid tissues. The avian experiments suggest that the thymus is responsible for the development of a population of cells with capacities for homograft reactivity and possibly also delayed sensitivity; the bursa on the other hand, is essential for the development of cells capable of producing immunoglobulins and humoral antibodies. The equivalent of bursa has not been found in mammals, although in some species, such as the mouse, it is possible that the thymus is responsible for the development of all the populations of cells with separate immunological reactivities. It has been suggested that the appendix may function as an equivalent of the bursa in the rabbit even though combined neonatal thymectomy and appendectomy does not completely inhibit the development of plasma cells and immunoglobulin synthesis.

The lymphocyte functions in the developing fetus have been the the subject of recent studies. The earliest appearance of small lymphocytes in the human fetus occurs in the peripheral blood at 7-8 weeks gestation (19 mm. body length) just before lymphocytopoiesis begins in the thymus. The circulating lymphocytes of these embryos were morphologically similar to adult small lymphocytes and comprised of 75 % of total lymphocytes.

According to Valdes Dapena, the thymic primordium arises from the 3rd pharyngeal pouch at 6 weeks of gestation when it was a whole epithelial structure.[54] During week 7, epithelial cells from the reticular network and lymphocytes appear within this by migration from the blood via the surrounding mesenchyme. By the middle of week 8, the thymus has differentiated further and medulla and cortex can be seen. Small lymphocytes can be detected in the thymus about the end of the second month of gestation in fetuses of 22-35 mm body length. The small lymphocytes that pass through the thymus are limited in their differientiative capacity in such a way that may only operate in cellular immunity.[54]

The spleen enlarge is first observed on week 5 of gestation. Small cells appear within the reticular framework and are later transformed to all the various cell types normally found in the bone marrow lymphocytes and are first seen definitely in the spleen from the end of 3 rd month of gestation.

Van Furth et al. reported that splenic pulp of fetuses of about 13-20 weeks of gestation only showed aggregates of large pyroninophilic cells (probably blast cells) and few lymphocytes.[19] In older fetuses

(up to 31 weeks gestation) both the amount of white pulp and the numbers of lymphocytes increased by 21 weeks. Spleen cells can synthesize trace amounts of M and G globulins in vitro.

According to Smith, in the spleen no germinal centers can be observed until shortly after birth.[55] At birth, there is chiefly red pulp with sleeves of lymphocytes around the terminal arteries and few primary follicles. These follicles grow both in size and number and are further differentiated by the 2nd month of life. By 5 months of age, there are a large number of both primary and secondary follicles with formation of clearly defined germinal centers and a few plasma cells.

Cornes found the mean number of Peyer's patches containing more than 5 follicles increased from 59 (at 24-29 weeks of gestation) to 100 at 0-10 weeks after birth, remained at 160 from 3-72 months and rose to 239 at 12-14 years of life. After 20 years of age, there was a decrease in the number of Payer's patches.[56]

We would also like to mention briefly our recent work on the onset of lympocyte function in the developing human fetus studied by culturing in vitro lympoid cells obtained from spleens and thymus glands of 16 fetuses of 12 to 28 weeks gestational age. The response of these cells to phytohemagglutinin was determined by measuring the incorporation of H_3 Tdr into DNA. In addition each organ was examined histologically. It was found that PHA responsive lymphocytes were present in the thymus at 12 week gestational age and in the spleen 2-4 weeks later (14 to 16 week gestational age). All thymuses examined had demarcated into lymphoid cortex and medulla. In the spleens, the onset of PHA response correlated with the appearance of small lymphocytes. Thymic cells from two fetuses of 15 and 16.5 weeks gestational age showed a vigorous response to allogeneic lymphocytes in mixed leukocyte cultures.[57] This mixed lymphocyte culture response of the thymocytes have also been reported in fetuses between 12 and 24 weeks by others.[58-61]

Hayward and Soothill reported the PHA-induced cytotoxicity of human fetal thymus lymphocytes to Cr^{51} labelled chicken red cells in fetuses between 16 and 26 weeks of gestational age.[58] They also found that the percentage of beta galactosidase binding cells in human fetal thymus cells seem to be falling by the increase in the gestational age.[58] The binding was inhibited by anti-K sera in two experiments.

Stites et al. examined 13 human fetuses of 11-19 weeks of gestation for the development of rosette-forming cells (RFC) with sheep red blood cells (SRBC).[62] RFC were found in the human fetal thymus as early as 11 weeks of gestational age. The number increased to 65 % around

the 15th-16th week. The percentage of RFC in the bone marrow was very low. After the 15th-16th week of gestation a slight decrease was noted in the percentage of RFC in the thymus. At the same time RFC appeared outside the thymus, suggesting a migration from the thymus.[62]

Summary

Phylogeny and Ontogeny of the immune response and the related literature is reviewed.

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