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Overview of immune system development in the dog: comparison with humans

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Dogs play an important role in toxicology because of their importance as a large animal, pre-clinical model for evaluating potential toxicity in human drug development including the effects of investigational drugs on the immune system. The purpose of this paper is to review the development of the canine immune system during the fetal, neonatal and postnatal periods and to compare it with that of the human immune system. Unlike rodents, the development of the canine immune system shares many similarities to that of the human. In both dogs and humans, the immune system, including the mucosal immune system, is fully developed before birth although the maturity of the immune response may continue into the postnatal period. *Human & Experimental Toxicology* (2002) **21**, 487–492.

Key words: canine; immune system; lymphocytes; ontogeny

Introduction

The dog has long been an important research model in two major areas. Dogs play an important role in the investigation of new drugs since they are one of the major models used in toxicity trials, including the effects of investigational drugs on the immune system. Historically, the dog has been a valuable model for bone marrow transplantation, with many of the advances made in the dog being directly transferable to human clinical bone marrow transplantation protocols. More recently, the dog has become an important model in the study of primary immunodeficiency disease. For example, the determination of the immunologic defect in X-linked severe combined immunodeficient (XSCID) dogs helped lead to the discovery of the gene responsible for both human and canine XSCID.

Since dogs are relatively outbred, share the same environment as humans, and develop many of the same immunologic diseases, they represent an ideal large animal model in which to study the immunology and pathogenesis of these diseases in a compressed period of time. Figure 1 illustrates the comparison of biologic aging between dogs and humans.[1] In the past, the major limitation of the use of the dog as an experimental model in immunologic research has been the paucity of immunologic reagents available to dissect the canine immune system. Over the past few years, great strides have been made in the development of these reagents.

Ontogeny of the immune system

The dog is a multiparous animal with a gestation period of 60–63 days. The following is a brief description of the fetal development of lymphoid organs in the dog.[2–8] Between days 27 and 28 of gestation, the primordia of the spleen and thymus are evident. On day 35, the thymic primordium descends from the cervical region into the anterior thoracic cavity. At this time, it is composed of epithelial lobules and mesenchymal stroma only. Between days 35 and 40, the thymus becomes actively lymphopoietic and shows corticomedullary demarcation. Hassall's corpuscles appear between days 38 and 40. By day 45, the thymic microenvironment has assumed its normal postnatal histologic appearance. Lymphocytic infiltration of lymph nodes and the spleen with evidence of T cell-dependent zones is evident between days 45 and 52. During this same time, the bone marrow becomes heavily cellular and contains abundant hematopoietic stem cells. Peyer's patches are present in the small intestine between days 45 and 55. By days 60–63, prominent postcapillary venules have developed in the peripheral lymphoid tissues. Germinal centers and plasma cells appear in the spleen and lymph nodes shortly after

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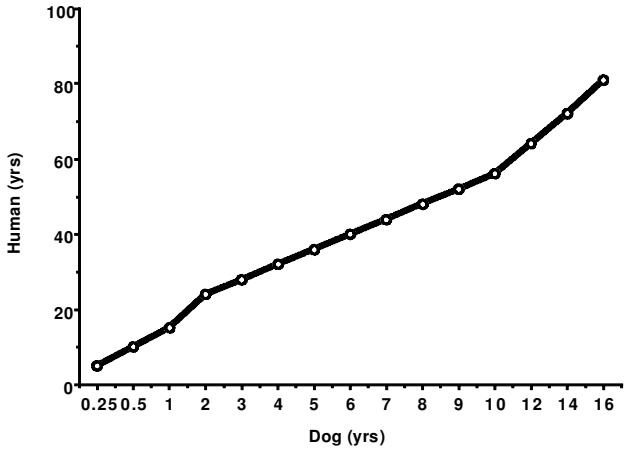

Figure 1 Comparison of biologic aging in dogs and humans

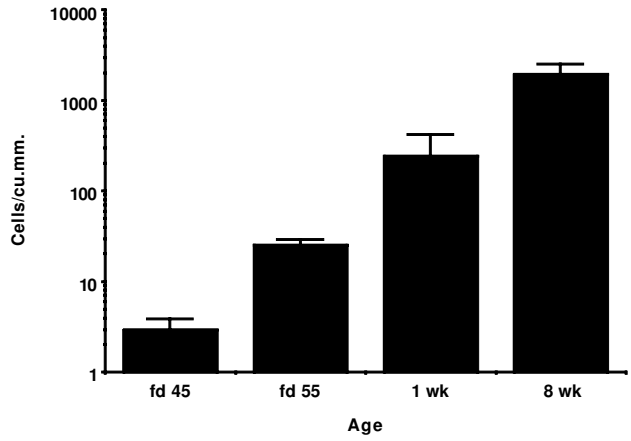

Figure 3 Thymocyte cellularity in the canine fetal and postnatal thymus (fd=fetal day)

birth. The thymus undergoes rapid postnatal growth and reaches maximum size at one–two months of age as percentage of body weight, and at six months of age in absolute terms.

Fetal and postnatal thympoiesis has only recently been evaluated in the dog (Refs. [8,9], Felsburg *et al.*, manuscript in preparation). Figure 2 illustrates that normal thympoiesis is occurring by day 45 of gestation with the distribution of thymocyte subsets virtually identical to that of the postnatal thymus. The only major difference between the fetal and postnatal thymus is its cellularity (Figure 3).

Our knowledge of the ontogeny of immune responses in the dog is limited and is summarized in Table 1.[8–14] Although fetal dogs are capable of responding to various antigens, it is generally considered that dogs become immunologically mature close to, or at, birth. The fact that neonatal dogs possess a functional humoral immune system was demonstrated by Jacoby *et al.*[10] In this study, colostrum-deprived, gnotobiotic puppies were vaccinated within the first 24 hours of birth with the T cell-dependent antigen, bacteriophage ϕX174. All the neonatal puppies developed a primary and secondary specific antibody response following immunization. The only difference between the fetal, neonatal, and adult groups of dogs was the magnitude of the response (Figure 4). These studies documented that neonatal dogs possess a functional B cell and T cell system at birth. Dogs immunized intranasally with a modified-live vaccine within the first week of life also develop a protective immune response, even in the presence of maternal antibody documenting the competence of the mucosal immune system.

Needless to say, the ontogeny of the human immune system is even more limited than the dog. Lobach and Haynes[15] have described the ontogeny of the human thymus during fetal development. The human thymus develops from the third pharyngeal pouch giving rise to the endodermal-derived thymic cortical epithelium and the third pharyngeal cleft giving rise to the thymic medullary epithelium. At seven weeks of gestation, the ectoderm and endoderm fuse to form an epithelial thymus. Between seven and eight weeks, prothymocytes from the fetal liver

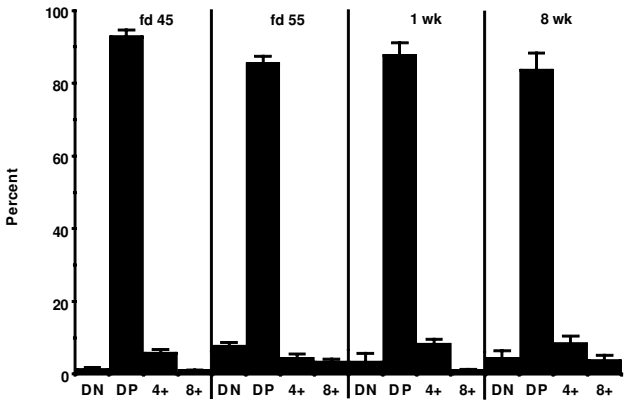

Figure 2 Thymocyte subsets in the canine fetal and postnatal thymus (fd=fetal day; DN=$CD4^-8^-$ thymocytes; DP=$CD4^+8^+$ thymocytes; 4+=$CD4^+8^-$ thymocytes; 8+=$CD4^-8^+$ thymocytes)

Table 1 Functional development of the immune system in fetal dogs

Day of gestation	*Immunologic function*
38–43	Appearance of CFU-g/m in fetal liver.
40	Respond to immunization with ϕX174.
45	Lymphocytes (spleen and lymph nodes) respond to PHA.
48	Respond to immunization with RBC.
50	Fetal thymocytes respond to PHA. Respond to immunization with *Brucella canis*.
Birth	Antibody response to KLH. Normal skin allograft rejection.

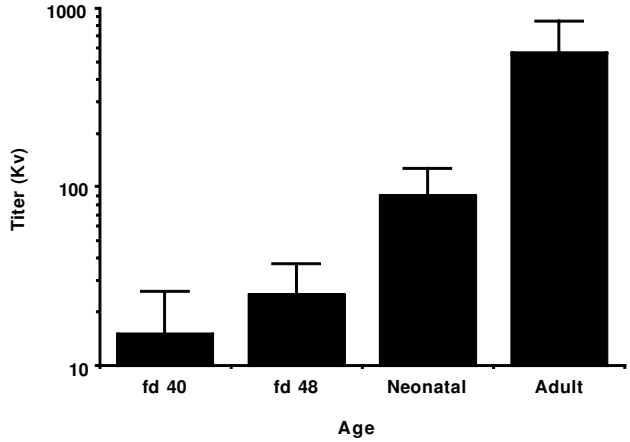

Figure 4 Antibody titers following immunization of fetal, neonatal and adult dogs with bacteriophage ϕX174 (fd=fetal day)

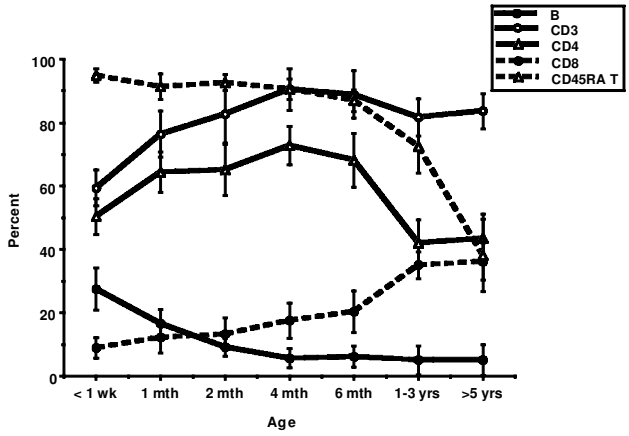

Figure 6 Age-related proportions of peripheral blood lymphocyte subsets

seed the thymus and thympoiesis begins. By 16–20 weeks, the development of the thymus is complete.

The seeding of the immune microenvironment during human fetal development was recently reviewed.[16,17] Hematopoiesis begins at three weeks of gestation in the chorion stalk yolk sac. At five weeks, the fetal liver is seeded with hematopoietic stem cells from the yolk sac and hematopoiesis begins in the fetal liver around six weeks of gestation. By eight weeks, progenitor cells seed the thymus, bone marrow, spleen and lymph nodes. Mature T cells seed the peripheral lymphoid organs between 11 and 12 weeks of gestation, and by 14–18 weeks, the spleen and lymph nodes contain the full complement of B and T cells. Because of the absence of antigenic stimulation in the fetus, there are no secondary follicles in the fetal spleen or lymph node. Peyer's patches are functionally mature by 20 weeks of gestation, but are quiescent until birth. Thymocytes respond to mitogenic stimulation at 12 weeks of gestation, and splenic T cells respond between 14 and 16 weeks.[18]

Neonatal and postnatal immune system development

In contrast to human neonates who receive the majority of their maternal antibody through placental transfer *in utero*, newborn puppies are essentially devoid of maternal antibody when they are born.[8] The placentation in the dog differs from the hemochorial placenta of humans in which the blood of the mother is in direct contact with the trophoblast permitting direct entry of maternal IgG into the fetal bloodstream. Dogs have an endotheliochorial placenta in which four structures separate the maternal and fetal blood – the endothelium of the uterine vessels and the chorion, mesenchyme (connective

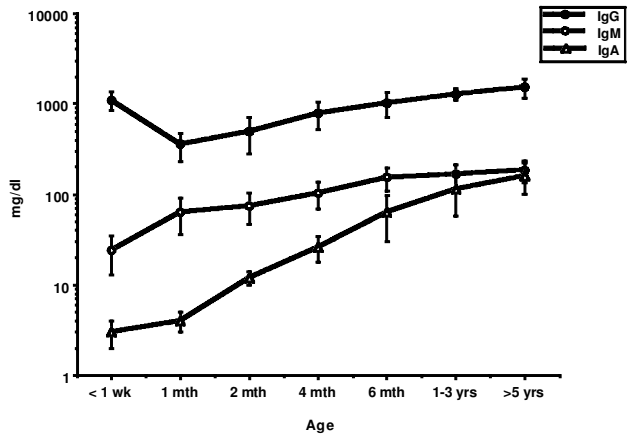

Figure 5 Age-related serum immunoglobulin concentrations in dogs

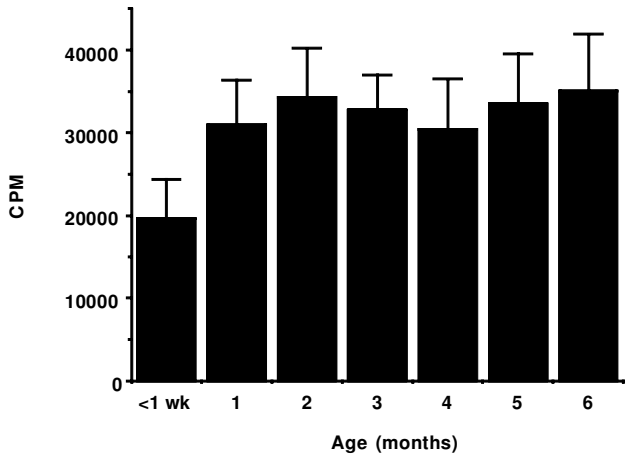

Figure 7 Age-related *in vitro* proliferative response of canine peripheral blood lymphocytes following stimulation with PHA (CPM=counts per minute)

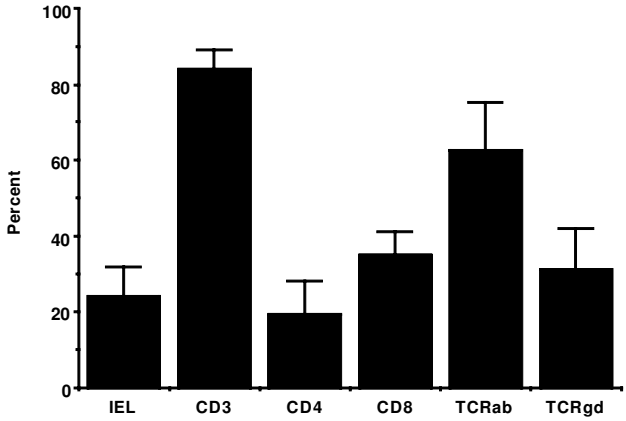

Figure 8 Proportion of canine IELs and various subsets of the IEL population

tissue), and the endothelium of the fetal tissues. These four layers of tissue between the maternal and fetal circulation in the dog limit the *in utero* transfer of maternal IgG to the fetus. Thus, only 5–10% of maternal antibody in the dog is obtained *in utero* through the placenta with the majority being obtained through colostrum during the first 24 hours after birth. The levels of serum IgG in newborn puppies that receive colostrum approach those levels found in adults. Since the half-life of maternal antibody in the dog is approximately 8.4 days, the average protection from maternal antibody in the neonate is between eight and 16 weeks.

Neonatal and postnatal immunoglobulin levels are dependent upon a variety of factors including age and environmental factors (infections, and others) with age being the most important. Depending upon whether the puppy received colostrum, IgG levels will be within the normal adult range in the neonatal period (Ref. [8], Felsburg, unpublished data). Following the decline of maternal IgG, there is a gradual increase in all three immunoglobulin classes (Figure 5). Normal adult levels of serum IgM occurs by two–three months of age. Serum IgG concentrations approach normal adult levels between six and nine months of age. As in other species, the synthesis of serum IgA lags behind the other isotypes and does not reach adult levels until approximately one year of age. This age-related development of serum immunoglobulins is similar to that observed in humans.[19]

The phenotype of the lymphocyte subpopulations in neonatal dogs differs significantly from that of adult dogs as illustrated in Figure 6 (Refs. [8,9], Felsburg *et al.*, unpublished data). During the first 16 weeks, there is a gradual decline in the proportion of peripheral B cells and an increase in the proportion of peripheral T cells to normal adult values. Thereafter, the proportion of B and T cells remains fairly constant throughout the life of the dog. Other age-related differences in lymphocyte subsets include considerably higher proportions of $CD4^+$ T cells during the first six months resulting in high CD4:CD8 ratios. After 10–12 months of age, the proportion of $CD4^+$ cells declines and the proportion of $CD8^+$ T cells increases to normal adult levels with normal CD4:CD8 ratios of 1:5–2.0. Lastly, during the neonatal and immediate postnatal period, the vast majority, greater than 90%, of the peripheral T cells are $CD45RA^+$ (naïve) T cells (Ref. [20], Felsburg *et al.*, unpublished data). After four months of age, the relative frequency of $CD45RA^+$ T cells declines such that only 40–50% of the peripheral T cells in adults are $CD45RA^+$. Very similar age-related changes are observed in healthy children and adults.[21,22]

Although the proportion of peripheral T cells in the neonatal dog is significantly lower than that in the adult, they are functionally competent as illustrated in Figure 7 by their ability to proliferate normally in response to mitogenic stimulation through the T cell receptor.

As discussed previously, dogs possess functionally mature Peyer's patches at the time of birth.[7,23,24] Neonatal dogs also possess intraepithelial lymphocytes (IELs) with a phenotype similar to that of adult dogs (Figure 8). The proportion of IELs and their phenotype is similar to that of human IELs including the predominance of $CD8\alpha\beta^+$ T cells in contrast to the predominance of $CD8\alpha\alpha^+$ T cells in rodents (Refs. [25,26], Felsburg *et al.*, unpublished data). As in the human, $TCR\alpha\beta^+$ T cells predominate in the skin of dogs in contrast to the predominance of $TCR\gamma\delta^+$ T cells in rodents.[27,28]

Table 2 Comparison of canine and human X-linked severe combine immunodeficieny with γc-deficient mice

	Human	*Dog*	*Mouse*
B cells	Normal, no class-switching	Normal, no class-switching	Absent
T cells	Low to absent	Low to absent	Low to absent
NK cells	Possible	Possible	Absent
Thymus			
Cellularity	??	1/300 Normal	1/25 Normal
Subsets	??	⇈DN ⇊DP	Normal
Postnatal T cell development	Yes (⇊ Normal)	Yes (25% Normal)	Yes (350% Normal)

Similarity to the immune system

The data presented in previous sections show that dogs, unlike rodents, but similar to humans, seem to be immunologically competent at, or before, birth. Like humans, the maturation of the immune response most likely continues postnatally due to the naivete of the neonatal immune system. Many of the descriptive parameters of the immune system in the dog also appear to be more similar to humans than rodents.

Further similarities of the canine immune system to the human system comes from studies involving XSCID that is caused by mutations in the common gamma chain (γc).[29] The γc is a common component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21,[29,30] cytokines that play an important role in lymphocyte development and function. Table 2 compares the effects of a mutated γc on human, canine and murine lymphocyte development and function.[9,20,31–43] It appears that species-specific differences exist concerning the role of the γc and its associated cytokines on lymphocyte development and function between the mouse and humans and dogs with the dog being virtually identical to the human.

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