Chapter 21

The perissodactyls: horses and their relatives

Plains zebra: Equus quagga. Courtesy of Fiona Tizard-Meyer.

The Perissodactyls diverged from the other mammalian orders in the great radiation that followed the K-Pg event around 65 mya. They diversified across what is now Eurasia and North America and the fossil record shows many extinct forms. They thrived during the Eocene about 38 mya years ago when climate change resulted in the massive expansion of grasslands. At that time, they were probably the most abundant herbivores in Laurasia. However, while they prospered for a time, they were eventually out-competed by the more efficient ruminants. In the late Eocene and early Oligocene about 35−30 mya, many failed to adapt to climate change during the cooler and dryer Oligocene epoch. Some subsequently migrated to Africa during the Miocene giving rise to modern rhinos and zebras. Thus, the great perissodactyl populations of North America including rhinoceroses and horses eventually died out. Some species survived however until the end of the ice ages, about 11,000 years ago. Their final extinction has been attributed to climate change but possibly also resulted, in part, from hunting by humans. The remaining species persisted over a much-reduced range in Asia and Africa. The tapirs remained jungle species and some migrated to South America where three species survive. The remaining wild perissodactyls are all endangered as a result of hunting and habitat destruction.

The odd-toed ungulates have reduced their weight-bearing digits to three as in rhinos, or one as in horses. Their nonweight-bearing toes are vestigial or absent. They are therefore classified as perissodactyls. Perissodactyls are exclusively herbivores. Unlike the ruminants, they retained their single stomachs but became hindgut fermenters to exploit the nutritional properties of the grasses. In effect, therefore, food that has already passed through the intestine is stored and further digested in the cecum. Thus, ideally extracting every possible calorie before it is excreted. The order includes 17 species classified into three families, the Equidae including horses, asses, and zebras, the Rhinocerotidae (rhinoceroses), and the Tapiridae (tapirs).

Comparative Mammalian Immunology. DOI: https://doi.org/10.1016/B978-0-323-95219-4.00007-1

The earliest evidence of horse domestication comes from the Volga-Don region of Western Eurasia around 5500 years ago. It is clear however that there were multiple subsequent domestication events and even feralization of some breeds [1]. As might be anticipated, there has been very little immunological research conducted on species other than the domestic horse. Surprisingly also, little is known about the immune system of the donkey, despite its importance as a mode of transport and beast of burden in many countries.

21.1 Reproduction and lactation

Horses use an epitheliochorial placenta. Six tissue layers separate the mother and her fetus. As a result, in order to provide sufficient oxygen and nutrition for the fetus, the entire surface of the placenta must be functional. In addition, only one fetus can usually be supported at a time. As another consequence, no immunoglobulins can cross the placenta, and foals are therefore born agammaglobulinemic.

The gestation period of the mare is about 340 days. Lymphocytes are seen first in the fetal thymus at about 60−80 days post-conception. They are found in the mesenteric lymph node and intestinal lamina propria at 90 days and the spleen at 175 days. Blood lymphocytes appear at about 120 days. A few plasma cells may be seen at 240 days. Graft-vs-host disease, a cell-mediated response, has developed in immunodeficient foals transplanted with tissues from a 79-day-old fetus. The equine fetus can respond to coliphage T2 by 200 days post-conception and Venezuelan equine encephalitis virus by 230 days. Like other large herbivores, the neonatal foal has a well-developed ileal Peyer's patch that may serve as a primary lymphoid organ and eventually involutes. Major B cell markers are detectable by 90−120 days gestation. *IGHM* and *IGLC* transcripts are expressed in the liver, bone marrow, and spleen at all ages. Immunoglobulin V region sequence diversity progressively increases as the fetus develops and as the foal develops into an adult. Thus, gene recombination and immunoglobulin class switching occur during equine fetal life despite the lack of antigenic stimulation [2,3]. As a result, newborn foals have detectable quantities of IgM and IgG in their serum, but IgE production in the horse does not begin until foals are 9−11 months of age [4].

In animals with a long gestation period, such as the horse, the adaptive immune system is fully developed at birth but cannot function at adult levels for several months. Thus, the production of IgG1, IgG3, IgG5, and IgA begin before or at birth and reach maturity at three months. Other antibody responses are much slower to develop. IgG4, and IgG7 production start shortly after birth and develop slowly over the first year of life. Similar delays are also seen in their T-cell responses [5]. For example, IFNγ production by $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells starts slowly, shortly after birth and gradually increases over the first year. In contrast, IL-4 is almost undetectable for the first three months of life. These findings, especially the slow development of type two responses, begin to explain how foals are very susceptible to some bacterial infections during their first months of life.

When a foal is born, it emerges from the sterile uterus into an environment where it is immediately exposed to a host of microorganisms. The complete development of adaptive immunity depends on antigenic stimulation. Thus, newborn foals are vulnerable to infection for the first few weeks of life. They need assistance in defending themselves at this time. This temporary help is provided by the mare's colostrum in the form of antibodies, cytokines, and possibly also T cells. The passive transfer of immunity from mare to foal is essential for survival.

Normal equine colostrum contains, on average, about 7000 mg/dL IgG but this can range from 3000 to 12,000 mg/dL. It also contains about 150 mg/dL 1gM and 300 mg/dL IgA. An average of about 100 g of IgG is produced per lactation in the mare [6]. Equine colostrum also contains about 20% IgA. There are significant amounts of IL-4 in a mare's colostrum which may serve to compensate for the very low level of IL-4 production in the young foal [5]. IL-13 has not been detected on the colostrum but IFN-γ is present in both colostrum and foal serum at birth. The chemokine IL-8 (CXCL8) is also present in significant amounts [7]. Equine colostrum is also rich in activated T cells. These may have a similar immune-stimulating function to that seen in pigs and cattle [8].

It is of interest to note that donkey foals are not totally agammaglobulinemic at birth. When born they have a significant concentration of IgG (8.97 $\pm$ 0.5 mg/mL) in their serum. In contrast, IgG concentrations in the serum of neonatal horse foals average 0.3 mg/mL [9].

21.2 Hematology

The leukocytes of the perissodactyls are generally unremarkable. The only exception is their eosinophils which contain many large granules (Fig. 21.1; Table 21.1) [10,11].

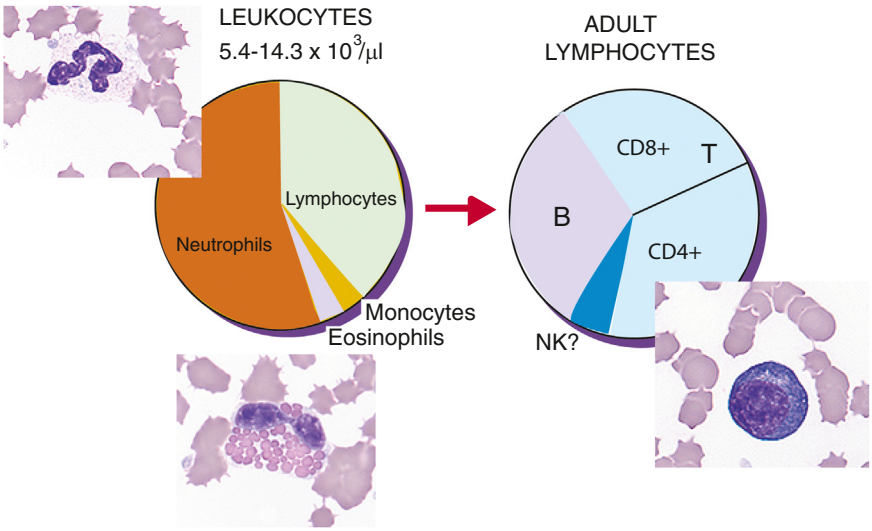

FIGURE 21.1 The blood leukocytes of the horse and the composition of adult equine lymphocyte populations. Note the large granules within the eosinophil. *Courtesy Dr. Mark Johnson.*

TABLE 21.1 Blood leukocyte counts in selected equine species.

	Horse*	Donkeys [10]	White rhino [11]
Total WBC $\times 10^3/\mu L$	4.3–15	10.1	14.5–16.1
Neutrophils (%)	28–83	60	49
Lymphocytes (%)	20–59	54	28
Monocytes (%)	1.5–10.5	3	12
Eosinophils (%)	0–9	3.7	15
Basophils (%)	0–2	<1	<1

*Courtesy of Dr. Karen Russell.

21.3 Innate immunity

21.3.1 Toll-like receptors

Equine toll-like receptors, TLR2, 3, 4, 5, 7, and 8 have been fully sequenced. Relative to human gene sequences they show 65%–77% nucleotide homology [12]. Thirteen SNPs have been found in equine TLRs 3, 7, and 8 while TLR4 shows restricted polymorphism.

21.3.2 Antimicrobial peptides

Horses are equipped with a diverse variety of bactericidal peptides. These include at least one lysozyme, five cathelicidins, nine defensins, one psoriasin, one hepcidin, and five possible equinins [13]. Equinins are a group of closely related proteinase inhibitors found in the granules of equine neutrophils as well as in their tracheobronchial secretions. They can inhibit microbial proteinase K and subtilisin and some have antibacterial and antiviral functions [14]. For example, they can kill *Streptococcus zooepidemicus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. They are also active against equine herpesvirus 2.

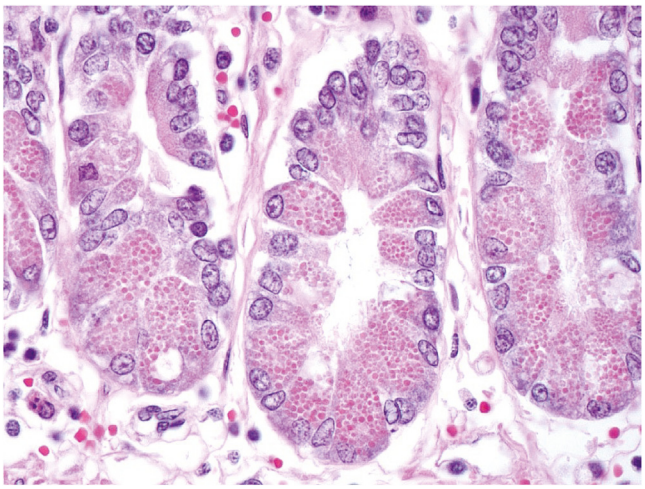

FIGURE 21.2 Paneth cells in the equine small intestine are a major source of intestinal defensins in this species. They are filled with large eosinophilic granules. Original magnification x 60. *Courtesy Dr. Brian Porter. From Tizard IR. Veterinary immunology. 10th ed. Saint Louis: Elsevier, 2016.*

Horses possess multiple beta-defensins that are ~60% identical to those in other mammals [15]. For example, equine beta-defensin-1 is 69.5% identical to porcine BD-1. They are expressed in many different tissues including the heart, liver, lungs, pancreas, and especially the Paneth cells of the gastrointestinal tract. (Fig. 21.2) A defensin gene cluster is located on equine chromosome 27q17. Analysis has identified nine possible defensin genes [16]. Six are very similar in sequence to human BD-4. Ten beta-defensin pseudogenes have also been identified.

Horses also possess multiple alpha-defensin genes. Thus, there are 38 gene transcripts of alpha-defensins in equine intestinal tissues and at least twenty may act as functional peptides [17]. However, no transcripts have been detected yet. If these transcripts are expressed, the horse would be the first eutherian outside the primates, lagomorphs, and rodents known to possess alpha-defensins [17].

21.3.3 Cytokines

Foals are interferon-gamma deficient at birth [18]. This may explain their marked susceptibility to certain bacteria such as *Rhodococcus equi* as well as viruses such as equine herpesvirus 1.

21.3.4 Interleukin-26

Interleukin-26 (IL-26) is a member of the IL-10 family. Its gene is located in a cluster bounded by the interferon-gamma gene on one side and the IL-22 gene on the other. The IL-26 gene is inactivated in horses and related equids such as zebras and donkeys as a result of a single base-pair frameshift. Since IL-26 is a stimulator of T cells and promotes inflammation and autoimmunity in other mammals, this inactivation may explain, in part, why some aspects of acute inflammation differ in horses. It is also of interest to note that the IL-26 gene has been inactivated independently in other mammals such as the African elephant (*Loxidonta Africana*) and the European hedgehog (*Erinaceus europaeus*) as well as in rats and mice [19].

21.3.5 Natural killer cells

Horses possess natural killer (NK) cells that are cytotoxic for MHC- class I deficient target cells [20]. As in humans, equine NK cells play an important role in remodeling the placenta as it grows, and the fetus develops. As discussed in Chapter 2, the interaction zone between the placenta is a site both of immune activity and rigorous immune regulation. The horse is no exception and there are dense infiltrations of lymphocytes surrounding the endometrial cup structures in the horse placenta during early pregnancy [21]. It has been shown that there is a significant enrichment in the uNK cell populations in the mare's placenta during pregnancy.

21.4 Lymphoid organs

21.4.1 Thymus

Most of the thymus in the newborn foal is located in the anterior mediastinum ventral to the trachea and large blood vessels. It consists of two lobes divided into lobules held together by connective tissue together with some adipose tissue. The thymic lobes may extend as a chain of lobules up the neck as far as the thyroid gland. However, the cervical lobes are very variable. One lobe may have no cervical projection while the other may give off a bifurcating one. The two lobes are in contact. Only the thoracic part may persist into adulthood. When the thymus atrophies, a thin remnant remains in the anterior mediastinum. Other perissodactyls such as rhinos have a thoracic thymus only [22].

The overall histologic structure of the thymus is similar to that seen in other mammals with a distinct cortex and medulla. As the animal ages, the thymic parenchyma progressively shrinks but the extra-parenchymal component does not. An unusual feature is the presence of prominent nonlymphoid hematopoiesis including eosinopoiesis, erythropoiesis, mastocytopoiesis, and plasmacytopoiesis in the equine thymus [23]. Lymphatic vessels full of lymphocytes are especially prominent and presumably serve as the routes by which selected T cells to leave the organ once fully functional. The thymic structure has been well described in rhinos [22].

21.4.2 Spleen

The equine spleen has the largest blood storage capacity among mammals. In addition to an extensive red pulp, it has a very muscular capsule and trabeculae. The capsule is bilayered with the inner layer and the trabeculae composed of elastic fibers and smooth muscle cells. The red pulp is of the nonsinusal type [24].

21.4.3 Mucosal lymphoid tissues

The horse has well-developed lymphoid tissues in its pharyngeal region. Thus, there is a lingual tonsil located at the root of the tongue. There is also a prominent tonsil in the soft palate located on the oral side. This soft-palate tonsil consists of a central crypt surrounded by primary and secondary lymphoid follicles and interfollicular lymphoid tissue [25]. Horses have two pharyngeal tonsils in the dorsal nasopharynx at the end of the nasal septum. These are not well defined and difficult to see macroscopically. As in other species, there is diffuse lymphoid tissue and isolated follicles located around the opening of the auditory tube—the tubal tonsils. These are continuous with the pharyngeal tonsils [26]. The follicle-associated epithelium overlying these tonsils contains M cells. The central follicular areas are composed largely of B cells but the dome and parafollicular areas contain both $CD4^+$ and $CD8^+$ T cells [27].

The tonsils of several species of rhinoceros have been examined in detail [28]. In addition to a large faucial tonsil, they possess a laryngopharyngeal tonsil located within the pyriform fossa that appears to develop from the third pharyngeal pouch in association with the thymus.

Because of its large size and the high volume of air that passes through the airways with every breath (100,00 L/24 hours) the horse may be considered more vulnerable to inhaled respiratory pathogens than smaller mammals. Bronchus-associated lymphoid tissue (BALT) is not present before birth in the foal. It has been suggested that species such as horses with a well-developed Waldeyer's ring in the pharynx and larynx have less need for bronchial lymphoid tissues. Surveys have shown that BALT is present in less than half of the horses surveyed. This of course may depend on the cleanliness of the inhaled air. Air from a dusty barn is very different from the air in an open pasture. When BALT does develop it is located in bronchioles at bifurcations where inhaled material is most likely to impact [26].

Peyer's patches are present in both the jejunum and ileum and there are isolated lymphoid follicles in the large intestine. The Peyer's patches develop during gestation and 245−320 have been counted in the jejunum of the newborn foal. Many disappear as the animal ages and their numbers drop to 100−200. In the ileum, however, there is a large 20−35 cm lymph node present in the newborn. It increases in size until sexual maturity but eventually disappears in older horses.

21.5 The major histocompatibility complex

The equine MHC locus, as in other mammals, is divided into three regions encoding Class II, Class III, and Class I in the usual order. The equine MHC, designated ELA, is located on chromosome 20q14-q22 (Fig. 21.3).

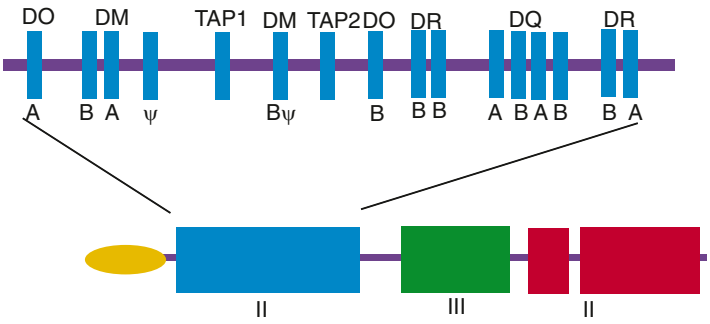

FIGURE 21.3 The organization of the equine major histocompatibility complex (ELA). Ψ = pseudogene.

21.5.1 The MHC class I region

Fifteen different MHC class I genes have been identified in the horse class I region. Of these seven appear to be functional [29]. Each of the class I genes encodes a protein chain with a leader sequence, three constant domains ($\alpha1-\alpha3$), a transmembrane domain, and two cytoplasmic domains. Most mammalian class I genes originated in the three duplication blocks, alpha, beta, and kappa, usually located within the class I region. However, while the equine class I genes are located within the three blocks, they are distributed throughout the MHC region. The alpha block is located at the *BNTL2* end of the class II region, The beta block is located at the junction of class III and I regions, while the kappa block is located between *GNL1* and *TRIM26* within the class I region. The horse beta block contains two putative nonclassical genes and four pseudogenes. In the horse kappa block, there are four classical genes, one putative nonclassical gene, and two pseudogenes. (In general, the horse class I region tends to resemble the pig class I except for the alpha block. This alpha block appears to be unique to the horse) [30].

21.5.2 The MHC class II region

The equine class II region is about 1.2 mb in size. It contains 35 gene loci. Of these, 20 have undisrupted open reading frames (ORF) [31]. Of the classical MHC class II genes in the ELA, there are six *DRB* loci of which three are functional. There are four pairs of *DQA-DQB* genes. Of the nonclassical MHC class II genes, four are functional—*DOA*, *DOB*, *DMA*, and *DMB*. The *DRB2*, *DRB3*, and *DQB3* genes have high sequence conservation [32]. In contrast, *DRB1* and *DQB1* are highly polymorphic.

When comparing these genes to other mammals, significant differences emerge. Thus, horses have four *DQA* and *DQB* loci while the cat has none. In the horse, the *DRB* genes are spread out across a region spanning 0.7 mb whereas, in the human, mouse, cat, pig, and cattle, they are clustered.

The organization of the ELA class II subregion of equids is similar across all species of the family tested. However, *DOB2* is not present in the genomes of the Asiatic asses, *Equus hemionus kulan*, and *E. h. onager* [32].

As in other related species, there is no evidence of any functional DP genes, although many, except the pig, have DP pseudogenes. The dog shows some similarities to the horse with a single functional *DRB* gene located on the reverse strand in a cluster with the *DRA* gene and an inverted *DRB* pseudogene. All the other laurasiatheria—dog, cat, pig, and bovine, have a single inverted *DRB* gene but the horse has five of them. Note that this inverted *DRB* gene is not present in humans or mice. This suggests that the inversion event occurred $\sim$80–100 mya following the separation of the Euarchontoglires but before the divergence of the laurasiatherian species. This eventually evolved into a separate inverted *ELA-DRB* gene cluster containing both functional genes and pseudogenes [31]. There are at least 17 *DRA* alleles, 14 *DRB* alleles, 27 *DQA* alleles, and more than 17 *DQB* alleles in the equine class II region [33].

The expression of MHC class II antigens on horse lymphocytes is determined by their MHC haplotype [34]. Thus, horses with haplotype H3 have lower levels of class II expression regardless of their age. (Foals express less MHC class II on their T cells regardless of haplotype). These class II antigens are expressed on both B and T cells.

At the boundary between the ELA class I and class III regions, there is a large, segmental duplication of about 710 kb. It consists of 11 repeated blocks, 10 of which contain an MHC class I-like sequence. The remaining block contains a full-length *BAT1* gene. A similar duplication is found in other Perissodactyls indicating that it is of ancient origin, probably over 20–55 my. Its persistence and transcription suggest that it is probably functionally important [35].

21.5.3 The natural killer receptor complex

In marked contrast to the other nonrodent mammals, the domestic horse and other members of the family Equidae employ multiple Ly49-like receptors as a result of significant expansion of the *LY49* gene locus, presumably by duplication [36,37].

Genomic analysis has shown the presence of six highly conserved polymorphic *LY49* genes in the NKC of horses, asses, and zebras [36]. Five of their products have a conserved immune-tyrosine-based inhibition motif (ITIM) and one has arginine in its transmembrane region. All five of these functional *Ly49* genes are present across the entire perissodactyl family including not only the equids but also rhinos and tapirs [36].

Several KIR-like sequences have also been detected in the equine genome although only one appears likely to encode a functional NK cell receptor [36]. There is also a nonfunctional KIR-immunoglobulin-like transcript fusion gene (*KIR-ILTA*) present in horses, donkeys, and plains zebra (*E. quagga*). There are two probable pseudogenes (*KIRP1* and *KIRP2*) with premature stop codons or frameshift mutations, and a *KIR3DL*-like sequence has also been identified. This protein contains a single nonmutated ITIM. This is a potentially functional *KIR* gene. These gene arrangements also appear to be present in tapirs and rhinos as well. Equine peripheral blood NK cells are active against diverse xenogeneic and allogeneic target cells. Thus, they are active against equine herpesvirus-1-infected equine embryonic kidney and embryonic lung cells [38].

21.5.4 Dendritic cells

Equine DCs have typical dendritic cell morphology—irregularly shaped large cells and prominent cytoplasmic processes. They express MHC class II, CD11, EqWC1, LFA1, and EqWC2 as would be anticipated from a cell type whose function is to present antigens to T cells. Different subsets have been identified based on their expression of MHC class II and other markers. Thus, dendritic cells from lymph nodes are CD1b positive but blood DCs are not [39]. DCs are present in equine lungs. They too express high levels of MHC I and CD44 but appear to be at an earlier developmental stage than blood DCs and have a significantly greater ability to take up antigens [40]. Equine pDCs have been characterized and shown to produce large amounts of IFN-α on stimulation with TLR9 agonists.

Dendritic cells can be readily derived from equine monocytes. They too are both MHC class II positive and CD14 positive. There appear to be two subpopulations that differ in size, but it is not known if they are functionally different [41]. Single-cell RNA sequencing has confirmed that three distinct dendritic cell subsets are present in equine blood including cDC1, cDC2, and plasmacytoid DCs [42].

21.6 B cells and immunoglobulins

Equine B cells express CD20 and MHC class II molecules as well as immunoglobulin antigen receptors. The majority of equine peripheral blood B cells are unusual in that they also express T-bet (T box expressed in T cells). T-bet is a T cell transcription factor. T-bet expression evolved long before the emergence of the adaptive immune system. It is normally expressed in helper T cells where it plays a role in regulating the expression of IFN-γ [43]. T-bet expression is usually associated with chronic infections and inflammation in humans and mice. In humans, when T cells proliferate and mutate in response to antigenic stimulation T-bet$^+$ memory B cells develop and promote B cell survival by regulating the transcription of the mature B cell receptor and thus regulate immunoglobulin class switching. It also drives the migration of some B cells to sites of inflammation. In horses, about half of these T-bet$^+$ cells express IgM, a quarter express IgG while the remainder express neither [43].

A significant increase in immunoglobulin diversity occurs during the long fetal life in the horse, especially through the last two-thirds of gestation despite the absence of stimulation by exogenous antigens. In the fetus, this diversity begins increasing about day 120 gestation and is focused on the third heavy chain complementarity determining region (CDR) within their V domains. In neonatal foals, the diversity increases in the second CDR as well. In adult animals, variation in the first CDR increases belatedly, although it is usually much less diverse than are CDRs 2 and 3. Some V, D, and J genes are used predominantly throughout life, and changes in relative gene usage do not occur as horses age [6].

21.6.1 Immunoglobulin heavy chains

The horse-heavy chain gene locus is located on chromosome 24q. This corresponds to human chromosome 14 where the IGH locus is located. Repeated duplication of the IGHG locus has resulted in the production of seven functional IgG subclasses in the horse (Fig. 21.4). All seven of these genes are expressed, in addition to the other four heavy chain genes, M, D, A, and E [44]. The seven IgG subclasses are designated IgG1 thru IgG7. (The previous nomenclature for IgG1 thru IgG4 was IgGa, IgGc, IgG(T), and IgGb. IgG6 was previously called IgG[B]). The former IgG(T) of the horse is now known to be a mixture of two subclasses IgG3 and IgG5.

IgG7 is closely related to IgG4 and likely resulted from a recent duplication of the IGHG4 gene. The relative expression ratio of IgG7 to IgG4 is 1:1.6. This duplication event has been detected in all horse breeds examined to date including both Thoroughbred horses and the isolated population of Icelandic horses [45]. Thus, the IGHG4/7 duplication event occurred more than 1000 years ago. IgG1, IgG4, and IgG7 are generally produced in response to intracellular infections while IgG3 and IgG5 are mainly produced in response to extracellular invaders. Horses have two IgG4 alleles ($IgG4^a$ and $IgG4^b$) and four IgE alleles (IgE^{1-4}). Horses also express IgM, IgD, IgA, and IgE. The equine IgM locus spans 1472 bp and encodes 451 amino acids. Its C_H3 and C_H4 exons are highly conserved.

The horse IGHD gene is located 5 kb downstream from IGHM. It spans about 9.1 kb and contains eight exons that are very similar in sequence to human IgD, namely—C_H1-H1-H2—C_H2-C_H3- S-M1-M2-. As in humans, no switch region has been detected upstream of the IGHD gene. IGHD is located very close to the IGHM gene, and it is possible that long pro-messenger transcripts might first form and are then subjected to alternative splicing or processing. IgD appears to be expressed at least at the mRNA level in equine B cells.

The equine IgE locus is similar to IgM in that there is no separate hinge region for IgE, and flexibility is conferred by C_H2. Interestingly, IgE concentrations in the equine bloodstream are about 1000-fold higher in normal horses than in normal humans [45] (Table 9.2).

There is only one isotype of equine IgA. The horse IgA locus contains three exons. These encode two constant domains and a single hinge region. The hinge region is an extension of the Cα2 domain. IgA is found in horse serum as well as in their mucosal secretions [44]. The IgA in mucosal secretions is produced in the form of secretory IgA. That is a dimer connected by a 17 kDa J chain. It is transported onto mucosal surfaces by the polymeric Ig receptor (pIgR), a type I transmembrane glycoprotein. The extracellular component of the pIgR is cleaved off to form secretory component which then protects the IgA against bacterial proteases. Both the equine J chain and pIgR have been characterized. The J chain contains four exons while pIgR contains 11. They have been mapped to equine chromosomes three and five respectively [46]. Many of the functions of IgA are mediated through its specific receptor, FcαR, otherwise called CD89. This receptor has been identified and characterized in the horse where it is located on chromosome 10 [47]. The genes encoding other classical Fc receptors are located on equine chromosome 5.

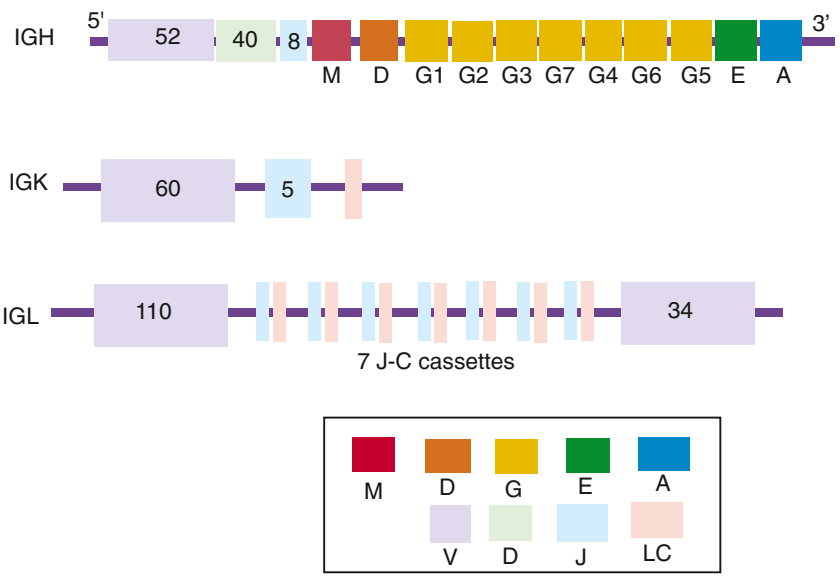

FIGURE 21.4 The organization of the equine immunoglobulin gene loci.

21.6.2 IGHV genes

Overall, the horse has 280 IGH genes while the white rhino (*Ceratotherium simum*) has 266. Rhinos have 69 IGHV genes. In the equine IGH locus, there are 52 IGHV, 40 IGHD, eight IGHJ, and 11 IGHC genes. The 40 identified D genes are among the largest number recorded in mammals, although guinea pigs have 41 and elephants have 87 [6]. The horse D segments range from 18 to 48 bp in length. This is longer than in humans (11−37 bp) but significantly shorter than in cattle. Horses therefore predominantly employ gene recombination.

The horse IGHV subfamilies belong to all three mammalian clans [48]. Thus subfamilies 1 and 5 belong to clan I; subfamilies 2, 4, 6, and 7, to clan II and subfamily 3 belongs to clan III. Note that clan III IGHV segments are found in all mammals Including camels, rabbits, platypus opossums, and pigs. (Fig. 9.6). In humans and mice, clan III is preferentially expressed during fetal life so these IGHV genes may play a role in the development of the foal's immune system. While the horse IGHV3 subfamily belongs to clan III and has at least ten members, none appear to be functional.

Examination of the equine B cell repertoire indicates that horses prefer to use the IGHV2 subfamily found in 92.49% of B cells. The CDR3 extended loop structure also predominates in the horse [49].

21.6.3 Immunoglobulin Light chains

The equine kappa light chain locus contains 60 IGKV, five IGKJ, and one IGKC gene. It is arranged in a single cluster of 820 kb. The ratio of Vλ to Vκ genes agrees with the preponderance of lambda chain use in horse serum although the ratio in serum is 13:1. Thus lambda light chains constitute ∼92% of the antibody repertoire in horses [6].

The lambda light chain locus IGL, contains seven IGLC genes, each preceded by a single IGLJ gene and 144 IGLV genes divided into two clusters both upstream and downstream of the J-C cluster. They extend for 1310 kb on chromosome 8. Each of the J segments is associated with a constant gene [6]. Horses preferentially use IGLV8 which is used in 82.5% of B cells.

The two clusters of IGLV genes are arranged in opposite transcriptional orientations. Within each cluster, there are both functional genes and pseudogenes. There are many more ORFs and pseudogenes than functional IGLV genes. However, allotypic and allelic variants are seen in IGLC1, IGLC5, and IGLC6/7. Two IGLV pseudogenes are also transcribed. The pseudogenes may also be used for gene conversion. V gene usage appears to not change throughout the animal's life although sequence diversity increases as animals develop from fetuses to adults [3].

21.7 T cells and cell-mediated immunity

There are two major T cell populations in the horse, $CD3^+$ $perforin^+$ (cytotoxic) cells, and $CD3^+$ $perforin^-$ (noncytotoxic) T cells [42]. Some of these cytotoxic cells are γ/δ positive. Horse lymphocytes express two species-specific proteins. EqWC1 is found on 70% of equine T cells, 30% of B cells, and 50% of granulocytes and could be a homolog of human CD90. A second specific protein, EqWC2 is expressed on granulocytes and most T cells. Both α/β and γ/δ T cells are widely distributed in multiple organs. The two cell types have a similar distribution, especially in epithelial and lymphoid tissues.

Treg cells expressing FoxP3 are present in the peripheral blood of horses. Their numbers are higher in horses under 6 years of age (2.7%) compared to older horses (1.5%) [50]. The proportion of $FoxP3^+$ cells is also much higher in foals than in their mothers. Not only that but they are functionally more suppressive [51].

In equine $CD8^+$ T cells both the α and β chains of the CD8 heterodimer are expressed in the cells from blood, thymus, spleen, mesenteric lymph node, and ileal intraepithelial lymphocytes [52]. However, there is also a subpopulation of CD8α/α cells present in the intestinal mucosa that account for up to a third of their intraepithelial lymphocytes.

21.7.1 T cell receptor genes

21.7.1.1 TRA/D genes

The equine TRA/D locus contains five unique TRAV gene segments in five different subfamilies, five distinct TRAJ genes that have conserved 3′ residues but diverse residues at their 5′ end, and a single TRAC gene (Fig. 21.5). The TRAC gene, when compared to other mammals, has highly conserved transmembrane and cytoplasmic domains. This conservation is almost certainly required to retain its ability to interact with the CD3 signaling peptides.

The equine TRD locus contains eight unique TRDV genes from seven subfamilies. These result in significant junctional diversity in the V-J region. It is of interest to note that the equine TRDV gene segments most closely resemble

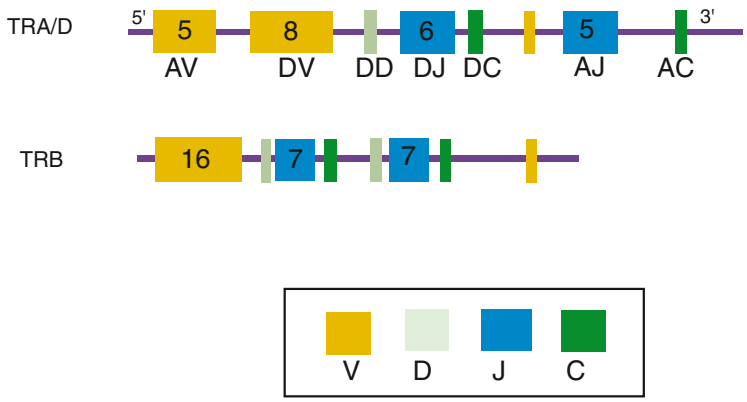

FIGURE 21.5 The equine TCR gene loci. No data is available as yet on the organization of the TRG locus except for the identification of two TRGC genes.

those in the sheep. Given that both are grazers exposed to a similar spectrum of intestinal parasites it may be speculated that these TRDV segments have been selected by the same antigenic stimuli. Horses have six, highly conserved TRDJ genes of which three are functional. The locus also contains a single TRDC gene that is homologous to that in other mammals except for its connecting peptide region [53].

21.7.1.2 TRB genes

The equine TRB gene locus contains 16 TRBV, 14 TRBJ, and two constant region genes. The TRBV genes are grouped into nine subfamilies based on 75% or greater similarities to the human sequences. The most common subfamily is TRBV2 which has four unique members out of five horses tested. Ten of the TRBV genes are full length while six are truncated pseudogenes. There are also obvious TRBV polymorphisms among the five different horse breeds tested (Thoroughbred, Arabian, Standardbred Quarter horse, and American miniature horse) [54]. Each of the constant genes TRBC1 and TRBC2 encode a peptide chain of 177 amino acids. The chains differ in nine amino acid residues of which four are located in the transmembrane domain and one in the cytoplasmic domain.

21.7.1.3 TRG genes

The horse has at least two TRGC genes. These have 77% nucleotide identity including four conserved cysteine residues and stop codons that are also found in humans, cattle, sheep, and mice. The duplication occurred a very long time ago. The two TRGC gene products differ most in their hinge region where TRGC1 has 18 more amino acids than TRGC2. It is speculated that these differences in their antigen receptors may affect the specific tissue distribution of equine γ/δ T cell populations [54]. Equine TRGV and TRGJ genes have yet to be characterized

21.7.2 Natural killer T cells and CD1

CD1d-restricted invariant natural killer T cells (NKT cells) are present in humans and mice, pigs and horses [55]. Using an invariant T cell α chain, these cells can recognize the characteristic glycolipid ligand α-galactosylceramide (α-GalCer) presented by a CD1d molecule. (Fig. 10.7). The presence of a functional CD1 system appears to be critical for horse health. As discussed in other chapters, the CD1 gene family is a group of nonpolymorphic MHC class I molecules that specifically present lipid antigens to T cells. In the horse, for example, they have been shown to present lipid antigens from the major equine pathogen, *R. equi* to cytotoxic T cells. The equine genome has been shown to contain 13 complete CD1 genes located in a 918 kb region on chromosome 5. Of these seven are homologous to human CD1a, two to CD1b, one each to CD1c and CD1d, and two to CD1e. It also contains five nonfunctional pseudogenes. This is one of the largest CD1 gene families yet recorded in mammals. Twelve of these CD1 molecules are expressed on antigen-presenting cells, including monocytes, macrophages, and dendritic cells. The polymorphism within the CD1 family is restricted to the antigen-binding α1 and α2 domains suggesting that multiple different lipid antigens can be presented and recognized. Lacking a tyrosine sorting motif, the eqCD1 molecules are predicted to colocalize with *R. equi* in intracellular vesicles [56].

Although horses possess functional NKT cells as well as a large CD1 gene family, they also differ in some respects from other mammals. For example, while α-GalCer is a potent NKT cell stimulator in most species examined, it is not however, an immunostimulatory NKT cell agonist in the horse [57].

References

[1] Brunson K, Reich D. The promise of paleogenomics beyond our own species. Trends Genet 2019;. Available from: https://doi.org/10.1016/j.tig.2019.02.006.

[2] Talmadge RL, McLaughlin K, Secor E, et al. Expression of essential B cell genes and immunoglobulin isotypes suggests active development and gene recombination during equine gestation. Dev Comp Immunol 2009;33:1027–38.

[3] Tallmadge RL, Tseng CT, King RA, Felippe MJB. Developmental progression of equine immunoglobulin heavy chain variable region diversity. Dev Comp Immunol 2013;41(1):33–43.

[4] Marti E, Ehrensperger F, Burger D, et al. Maternal transfer of IgE and subsequent development of IgE responses in the horse (*Equus caballus*). Vet Immunol Immunopathol 2009;127:203–11.

[5] Perkins GA, Wagner B. The development of equine immunity: current knowledge on immunology of the young horse. Equine Vet J 2015;47:267–74.

[6] Walther S, Rusitzka TV, Diesterbeck US, Czarny C-P. Equine immunoglobulins and organization of immunoglobulin genes. Dev Comp Immunol 2015;53:303–19.

[7] Mariella J, Castagnetti, Prosperi A, Scagliarini A, Peli A. Cytokine levels in colostrum and in foals' serum pre-and post-suckling. Vet Immunol Immunopathol 2017;185:34–7.

[8] Perkins GA, Goodman LB, Wimer C, Freer H, et al. Maternal T-lymphocytes in equine colostrum express a primarily inflammatory phenotype. Vet Immunol Immunopathol 2014;161:141–50.

[9] Turini L, Bonelli F, Nocera I, Battaglia F, et al. Evaluation of Jennies' colostrum: IgG concentrations and absorption in the donkey foals. A preliminary study. Heliyon 2020;. Available from: https://doi.org/10.1016/j.heliyon.2020.e04598.

[10] Burden FA, Hazell-Smith E, Mulugeta G, Patrick V, et al. Reference intervals for biochemical and haematological parameters in mature domestic donkeys (*Equus asinus*) in the UK. Equine Vet Educ 2016;28(3):134–9.

[11] Miller M, Buss P, Wanty R, Parsons S, et al. Baseline hematological results for free-ranging white rhinosceros (*Ceratotherium simum*) in Kruger National Park, South Africa. J Wildlife Dis 2015;51(4):916–22.

[12] Jungi TW, Farhat K, Burgener IA, Werling D. Toll-like receptors in domestic animals. Cell Tissue Res 2011;343:107–20.

[13] Bruhn O, Grotzinger J, Cascorbi I, Jung S. Antimicrobial peptides and proteins of the horse – insights into a well-armed organism. BMC Vet Res 2011;. Available from: https://doi.org/10.1186/1297-9716-42-98.

[14] Pellegrini A, Kalking M, Hermann M, Grünig B, et al. Equinins in equine neutrophils: quantification in tracheobronchial secretions as an aid in the diagnosis of chronic pulmonary disease. Vet J 1998;155:257–62.

[15] Bagnicka E, Strazlkowska N, Józwik A, Krzyzewsli J, et al. Expression and polymorphism of defensins in farm animals. Acta Biochim Polon. 2010;57(4):487–97.

[16] Looft C, Paul S, Regenhard P, Kuiper H, et al. Sequence analysis of a 212 kb defensin gene cluster on ECA 27q17. Gene 2006;376:192–8.

[17] Bruhn O, Paul S, Tetens J, Thaller G. The repertoire of equine intestinal α-defensins. BMC Genomics 2009;. Available from: https://doi.org/10.1186/1471-2164-10-631.

[18] Breathnach CC, Sturgill-Wright T, Stiltner JL, et al. Foals are interferon gamma-deficient at birth. Vet Immunol Immunopathol 2006;112:199–209.

[19] Shakhsi-Niaei M, Drogemuller M, Jagannathan V, Gerber V, Leeb T. IL-26 gene inactivation in Equidae. Anim Genet 2013;44:770–2.

[20] Vivieros MM, Antczak DF. Characterization of equine natural killer and IL-2 stimulated lymphokine activated killer cell populations. Dev Comp Immunol 1999;23:521–32.

[21] Noronha LE, Huggler KE, de Mestre AM, Miller DC, Antczak DF. Molecular evidence for natural killer-like cells in equine endometrial cups. Placenta 2012;33:379–86.

[22] Cave AJE. The thymus gland in three genera of rhinoceros. J Zool 2009;142(1):73–84.

[23] Contreiras EC, Lenzi HL, Meirelles MNL, Caputo LFG, et al. The equine thymus microenvironment: a morphological and immunochemical analysis. Dev Comp Immunol 2004;28:251–64.

[24] Udroiu I. Evolution of sinusal and non-sinusal spleens of mammals. Hystrix It J Mamm 2006;17(2):99–116.

[25] Casteleyn C, Breugelmans S, Simoens P, Van den Broeck W. The tonsils revisited: review of the anatomical localization and histological characteristics of the tonsils of domestic and laboratory animals. Clin Dev Immunol 2011;. Available from: https://doi.org/10.1155/2011/4/472460.

[26] Liebler-Torino E, Pabst R. MALT structure and function in farm animals. BMC Vet Res 2006;37(3):257–80.

[27] Kumar P, Timoney JF, Sheoran AS. M cells and associated lymphoid tissue of the equine nasopharyngeal tonsil. Equine Vet J 2001;33(3):224–30.

[28] Cave AJE. The rhinoceros faucial and laryngopharyngeal tonsils. J Zool Lond 1979;187:471–503.

[29] Tallmadge RL, Lear TL, Antczak DF. Genomic characterization of MHC class I genes in the horse. Immunogenetics 2005;57:763–74.

[30] Tallmadge RL, Campbell JA, Miller DC, Antczak DF. Analysis of MHC class I genes across horse MHC haplotypes. Immunogenetics 2010;62:159–72.

[31] Viluma A, Mikko S, Hahn D, Skow L, Andersson G, Bergstrom TF. Genomic structure of the horse major histocompatibility complex class II region resolved using PacBio long-read sequencing technology. Sci Rep 7:45518; doi:10.1038/srep45518

[32] Klumplerova M, Splichalova P, Oppelt J, Futas J, et al. Genetic diversity, evolution and selection in the major histocompatibility complex *DRB* and *DQB* loci in the family Equidae. BMC Genomics 2020;. Available from: https://doi.org/10.1186/s12864-020-07089-6.

[33] Fraser DG, Bailey E. Polymorphism and multiple loci for the horse *DQA* gene. Immunogenetics 1998;47:487–90.

[34] Barbis DP, Bainbridge D, Crump AL, Zhang CH, Antczak DF. Variation in expression of MHC class II antigens on horse lymphocytes determined by MHC haplotype. Vet Immunol Immunopathol 1994;42:103–14.
[35] Brinkmeyer-Langford CL, Murphy WJ, Childers CP, Slow LC. A conserved segmental duplication within ELA. Anim Genet 2010;. Available from: https://doi.org/10.1111/j.1365.2052.2010.02137.x.
[36] Futas J, Horin P. Natural killer cell receptor genes in the family Equidae: Not only Ly49. PLoS One 2013;. Available from: https://doi.org/10.1371/journal.pone.0064736.
[37] Takahashi T, Yawata M, Raudsepp T, et al. Natural killer cell receptors in the horse: evidence for the existence of multiple transcribed LY49. Eur J Immunol 2004;34:773–84.
[38] Chong YC, Duffus WPH, Hannant D. Natural killer cells in normal horses and specific-pathogen-free foals infected with equine herpesvirus. Vet Immunol Immunopathol 1992;33:103–13.
[39] Siedek E, Little S, Mayall S, Edington N, Hamblin A. Isolation and characterization of equine dendritic cells. Vet Immunol Immunopathol 1997;60:15–31.
[40] Lee Y, Kiupei M, Soboll Hussey G. Characterization of respiratory dendritic cells from equine lung tissues. BMC Vet Res 2017;. Available from: https://doi.org/10.1186/s12917-017-1240-z.
[41] Lopez BS, Hurley DJ, Giancola S, Giguère S, et al. Phenotypic characterization of equine monocyte-derived dendritic cells generated ex vivo using commercially available serum-free medium. Vet Immunol Immunopathol 2020;. Available from: https://doi.org/10.1016/j.vetimm.2020.110036.
[42] Patel RS, Tomlinson JE, Divers TJ, Van der Walle GR, Rosenberg BR. Single-cell resolution landscape of equine peripheral blood mononuclear cells reveals diverse cell types including T-bet + B cells. BMC Biol; doi:10.1186/s12915-020-00947-5
[43] Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. Nature Rev Immunol 2013;13:777–89.
[44] Wagner B. IgE in horses: occurrence in health and disease. Vet Immunol Immunopathol 2009;132:21–30.
[45] Wagner B, Miller DC, Lear TL, Antczak DF. The complete map of the Ig heavy chain constant gene region reveals evidence for seven IgG isotypes and for IgD in the horse. J Immunol 2004;173:3230–42.
[46] Lewis MJ, Wagner B, Irvine RM, Woof JM. IgA in the horse: cloning of equine polymeric Ig receptor and J chain and characterization of recombinant forms of equine IgA. Mucosal Immunol 2010;3(6):610–21.
[47] Morton HC, Pleass RJ, Storset AK, Brandtzaeg, et al. Cloning and characterization of equine CD89 and identification of the CD89 gene in chimpanzees and rhesus macaques. Immunology 2005;115:74–84.
[48] Sun Y, Wang C, Wang Y, Zhang T, et al. A comprehensive analysis of germline and expressed immunoglobulin repertoire in the horse. Dev Comp Immunol 2010;34:1009–20.
[49] Manso TC, Groenner-Penna M, Minozzo JC, Antunes BC, et al. Next-generation sequencing reveals new insights into gene usage and CDR-H3 composition in the horse antibody repertoire. Mol Immunol 2019;105:251–9.
[50] Robbin MG, Wagner B, Noronha LE, Antczak DF, de Mestre AM. Subpopulations of equine blood lymphocytes expressing regulatory T cell markers. Vet Immunol Immunopathol 2011;140:90–101.
[51] Hamza E, Mirkovitch J, Steinbach Marti. Regulatory T cells in early life: comparative study of CD4 + CD25 high T cells from foals and adult horses. PLoS One 2015; 1371/journal.pone.0120661.
[52] Tschetter JR, Davis WC, Perryman LE, McGuire TC. CD8 dimer usage on ab and gd T lymphocytes from equine lymphoid tissues. Immunobiology 1997;198:424–38 98.
[53] Schrenzel MD, Ferrick DA. Horse (*Equus caballus*) T cell receptor alpha, gamma and delta chain genes: nucleotide sequences and tissue specific gene expression. Immunogenetics 1995;42:112–22.
[54] Schrenzel MD, Watson JL, Ferrick DA. Characterization of horse (*Equus caballus*) T-cell receptor beta chain genes. Immunogenetics 1994;40:135–44.
[55] Van Beeck FAL, Reinink P, Hermsen R, Zajonc D, et al. Functional CD1d and/or NKT invariant chain transcript in horse, pig, African elephant and Guinea pig, but not in ruminants. Mol Immunol 2009;46:1424–31.
[56] Dossa RG, Alperin DC, Hines MT, Hines SA. The equine CD1 gene family is the largest and most diverse yet identified. Immunogenetics 2014;66:33–42.
[57] Dossa RG, Alperin DC, Garzon D, Mealey RH, et al. In contrast to other species, α-galactosylceramide (α-GalCer) is not an immunostimulatory NKT cell agonist in horses. Dev Comp Immunol 2015;49:49–58.