

THE IMPORTANCE OF THE AVIAN IMMUNE SYSTEM AND ITS UNIQUE FEATURES

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INTRODUCTION

The avian immune system provides an invaluable model for studies on basic immunology. Birds and mammals evolved from a common reptilian ancestor more than 200 million years ago and have inherited many common immunological systems. They have also developed a number of very different and, in some cases, remarkable strategies. Due to their economic importance, and the ready availability of inbred lines, most avian research immunology has involved the domestic chicken, *Gallus gallus domesticus*. A remarkable consequence of this research has been the seminal contributions it has made to understanding fundamental immunological concepts, especially the complete separation of developing bursa (B)- and thymus (T)-dependent lymphocyte lineages. Some of the observations were made by chance, while others resulted from painstaking work which took advantage of special avian features, for instance the ease of access to, and precise timing of, all the stages in embryonic development. Some of the avian findings were described before being recognized as important and subsequently explained in mainstream immunology. The story of avian immunology is fascinating and by no means complete, as there is still the need for explanations of a number of unique features and the different strategies adopted by the avian system. In this chapter some of the “firsts”, rightly attributed to avian immunology, are described and the importance of further studies in avian immunology highlighted.

THE CONTRIBUTION FROM AVIAN LYMPHOCYTES

The advent of modern cellular immunology, and the fundamental role that lymphocytes play, is generally credited to the 1950s and 1960s, when lymphocyte function became an active subject for research (Burnet, 1971). The immunological significance of lymphocytes emanated from

some seminal studies carried out by Gowans, Chase and Mitchison, Simonsen and their contemporaries (see Gowans, 1959; Burnet, 1971). In elegant experiments using laboratory mammals, these workers demonstrated using cell transfers that lymphocytes are essential for generating immune responses and retaining memory of previous exposure to an antigen. However, evidence that lymphocytes play such a key role in protection against infection and in tumour rejection had been in existence for almost 40 years (Murphy, 1914a, b, 1916, 1926), though little attention had been paid to it (Silverstein, 2001). This was almost certainly because, at the time that they made, the observations could not be properly explained and, possibly, because the experimental animal involved was the chicken.

Between 1912 and 1921, James Murphy, an experimental pathologist working at the Rockefeller Institute for Medical Research in New York, performed a series of remarkable experiments using chickens and their embryos to study the growth and rejection of tumour grafts. His experiments appeared to prove beyond question that the lymphocyte is the active component in tissue graft rejection, in protection against infection and, by implication, in innate and acquired immune responses (Silverstein, 2001). Murphy (1914a) observed that the fragments of rat tumours would not grow in the adult chicken, just as they did not grow in other species (xenogenic rejection). However, they could be grown on the chorioallantoic membrane (CAM) of developing chick embryos, although only up to about 18 days incubation. In older embryos tumour grafts were rejected, just as they were if grafted onto the newly hatched chick or an adult bird. Interestingly, Murphy (1914a) observed that the grafts which grew on embryos could be transferred to fresh embryos without any evidence of them being altered. They also retained their tumorigenic capacity if re-grafted onto a rat. Murphy (1914a) commented that these cellular changes occurring when living tissue is grafted onto an unsuitable host are the same, regardless of the type of host resistance, be it natural resistance because of species differences (allogeneic or xenogeneic rejection) or acquired immunity due to recovery from a tumour implanted earlier. The histological picture consisted of oedema surrounded by fibroplasia in the host tissue, the budding out of blood vessels and infiltration of surrounding host tissues with small lymphocytic cells. The inevitable consequence was that cells in the graft died fairly quickly leaving only a scar. These were quite profound though, at the time, unappreciated observations.

Murphy (1914b) also performed a series of elegant experiments using adult chicken tissues co-grafted onto the CAM with fragments of rat tumours. He observed that chicken tissues containing an abundant supply of lymphocytes, such as the spleen or bone marrow, caused tumour grafts to be rejected; whereas these were not rejected if the co-grafted tissue lacked a rich supply of lymphocytes. Later on, Murphy (1916) showed that after grafting fragments of adult chicken spleen onto the CAM of a 7-day embryo, the embryo's own spleen became grossly enlarged (splenomegaly). This is the first published record of a graft-versus-host response (GvHR). Much later, it was explained by Simonsen (1957) as the immunologically competent lymphocytes of the adult responding to mismatched major histocompatibility complex (MHC) molecules expressed by the embryonic cells. The embryonic cells were recognized as foreign causing the adult cells to replicate and destroy the embryonic host's lymphocytes. Graft-versus-host disease, in which allogeneic bone marrow transplants recognize the tissues of the graft recipient and cause severe inflammatory disease, is a major problem for immunosuppressed recipients receiving bone marrow transplants. The phenomenon became a major concern with the introduction of human bone marrow transplantation. Nonetheless, the phenomenon was first described using chick embryos (Murphy, 1916).

THE CONTRIBUTION OF THE BURSA OF FABRICIUS

Without doubt, the most significant contribution that avian immunology has made to development of mainstream immunology was in delineating the two major arms of the adaptive immune system. As already pointed out, in the 1960s the significance of lymphocytes was just becoming appreciated and it was generally accepted that there are two types of adaptive

immune responses: humoral responses involving antibodies and cellular responses mediated by macrophages and lymphocytes. Since antibodies are produced by plasma cells, which themselves are derived from lymphocytes, it was not understood how such lymphocytes could be of the same type as those cells involved in cell-mediated functions. The bursa of Fabricius, an obscure sac-like structure attached to the proctodeal region of the bird's cloaca (Plate 1.1), played a crucial role in unravelling this problem.

The cloacal bursa, takes its name from Hieronymus Fabricius of Aquapendente (1537–1619), also known as Girolamo Fabrizi d' Acquapendente (Fig. 1.1). He was Professor of Surgery at the University of Padua, Italy from 1565 to 1613 (Adelmann, 1942) and by all accounts a brilliant anatomist, embryologist and teacher. For his pioneering work he was later credited in Italian medical science as the “Father of Embryology”. Fabricius not only carried out dissections on human cadavers but also extended his anatomical studies to other species, providing most beautiful detailed drawings of his work. From his observations of avian anatomy he surmised that the cloacal bursa, a hollow structure connected by a duct to the proctodeal region of the cloaca in the hen, most likely acts as a receptacle for storing semen:

Since the sac is pervious, so that there is an open passage from the anus to the uterus itself and another from the uterus to the sac, that is, above and below, and since it is closed at the other end, I think it is the place into which the cock introduces and delivers semen so that it may be stored there (from Adelmann, 1942).

This is not the case, however, but the role of the cloacal bursa continued to puzzle researchers over the following 350 years. Some surmised that, since the bursa of Fabricius regresses with sexual maturity, its size having an inverse relationship with the size testes and the adrenals, it must be some sort of endocrine or lymphoid gland associated with growth and sexual development (Glick, 1987).



FIGURE 1.1 Hieronymus Fabricius of Aquapendente, Professor of Surgery at the University of Padua from 1565 to 1613. Fabricius was the first to describe the bursa, which is now known to be a primary lymphoid organ, attached by a duct to the proctodeum in birds and essential for the development of bursal-derived (B) lymphocytes and the avian antibody repertoire.

Over the years many investigated the function(s) of the bursa including one young researcher, Bruce Glick, working at the Poultry Science Department at Ohio State University, USA. Glick surgically removed bursas from young chicks to investigate the effect on growth. By chance, after one experiment was concluded a colleague, Timothy Chang, asked if he could use some of the birds for a class demonstration on antibody production. A group of the chickens was injected with *Salmonella* spp. “O” antigen but 1 week later, when the class carried out tests with blood and antigen, there was no evidence of agglutination. Chang, somewhat perplexed, reported the failure to Glick who was able to identify the non-responder chickens as those that had been bursectomized. At the time they did not seem to fully appreciate the singular importance of this finding (Glick, 1987), but were able to confirm their initial observations in further experiments and wrote up a paper entitled: “The role of the bursa of Fabricius in antibody production.” This was submitted to *Science* but rejected on the grounds that further elucidation of the mechanisms was necessary before the paper could be accepted for publication (see Glick, 1987). The paper was subsequently submitted to *Poultry Science* (Glick *et al.*, 1956), where, for a time, it failed to draw much attention. Several years passed before the significance of the work was properly appreciated and mainstream immunologists took an interest in the chicken’s immune system. It was eventually concluded that the avian bursa must be essential for antibody-mediated immunity, whereas the thymus, which also undergoes involution during sexual development, is necessary for cell-mediated immunity (Szenberg and Warner, 1962; Warner and Szenberg, 1962; Warner *et al.*, 1962). Almost a decade after Glick and Chang’s initial observations (Glick *et al.*, 1956), Cooper *et al.* (1965) published their seminal paper on the delineation of the bursal and thymic lymphoid systems in the chicken. These workers proposed that, because of the similarities in the lymphoid tissues and immune systems of birds and mammals, a mammalian equivalent for the bursa of Fabricius must exist and provide a source of B-dependent lymphocytes to make antibodies. Later this bursa equivalent was identified as bone marrow. The division of the adaptive immune system into B- and T-dependent compartments has remained a central tenet of immunological thinking ever since. The term B lymphocyte is derived from “bursa-derived lymphocyte” in honour of that peculiar avian lymphoid structure which provided the original evidence.

Gene Conversion and the Bursa

Antibodies recognize specific conformational molecular shapes on their target through the immunoglobulin (Ig) variable region. Antigenic shapes are legion so an immunocompetent individual must be capable of generating an antibody repertoire with a huge number of Ig specificities (Janeway *et al.*, 2001). Different B cells produce Ig molecules of different specificities and each B cell is capable of producing only one Ig specificity. In man and mouse the antibody repertoire of B cells is generated by a process known as Ig gene rearrangement, which is ongoing throughout life. In the case of the Ig light chain, genes that encode the variable region (V_L gene segment), a joining region (J_L gene segment) and the constant region (C_L gene segment) are spatially separated by non-coding segments in germline DNA (Plate 3.4). Non-coding segments are spliced to allow the joining of $V_L J_L$ regions in genomic DNA, while excision of the non-coding section in the RNA transcript allows the $V_L J_L C_L$ regions to combine permitting translation of a functional Ig light chain molecule (Tonegawa, 1983). In the case of the Ig heavy chain a further diversity (D) gene is involved, making a $V_H D J_H$ rearrangement, otherwise the process is similar. Since multiple copies of V, J and D genes exist in the mammalian Ig locus: (1) random recombination of the different gene segments; (2) different combinations of heavy and light chains paired together; diversity introduced at the joints between the different gene segments by the variable addition and subtraction of nucleotides; and finally (3) point mutations introduced into the rearranged sequence diversifying these region further (somatic hypermutation), allow for a vast ($\sim 10^{11}$) antibody repertoire to be generated (Janeway *et al.*, 2001).

By contrast the cluster of genes encoding the chicken Ig light chain has only a single copy of the functional V_L and J_L genes (Reynard *et al.*, 1985). Hence diversity due to $V_L J_L$ joining is very limited and the effects of VJ rearrangement are minimal (a detailed description can be

found in Chapter 4). Likewise with the Ig heavy chain locus, the presence of single functional V_H and J_H genes means that little diversity can be generated through V_HDJ_H rearrangement. However, clusters pseudogenes, upstream of the heavy and light chain Ig loci have a critical role in the generation of chicken antibody diversity. By a process known as somatic gene conversion, V_L and V_H sequences are replaced with pseudogene sequences. An enormous amount of diversity is generated by substantial diversity in the hypervariable regions of the donor V pseudogenes and somatic gene conversion events accumulate within single functional V_L or V_H genes. Perhaps, chickens represent the extreme situation with only one functional V_L gene while other species such as the duck have up to four functional V_L genes, although they still use gene conversion to introduce variability. It seems that, uniquely, birds rely on somatic gene conversion for generating an antibody repertoire which is the equal of that in immunocompetent mammals. Interestingly, it has recently been shown that if gene conversion is blocked in chicken B cells then somatic hypermutation occurs instead (Arakawa *et al.*, 2002). It has also been observed that gene conversion is not just limited to birds. Gene conversion also occurs in rabbits (Becker and Knight, 1990), pigs and other mammalian species, though none appear to rely on it as the exclusive means of generating the antibody repertoire.

The striking fact about avian somatic gene conversion is that it only occurs in the bursa of Fabricius (see Chapter 4). For instance, if the bursa is destroyed early in development (60h), then those chicks that hatch produce only non-specific IgM and are unable to mount specific antibody responses. In other words, they do not have an antibody repertoire and are incapable of eliciting typical responses or isotype switching to produce IgG or IgA. Whereas, if the bursa is removed much later during embryonic development, but before 18 days when the B lymphocytes have begun to migrate from the bursa into the peripheral lymphoid tissues, then the hatched chicks lack circulating Ig and are incapable of eliciting specific antibody responses. We know that pre-bursal stem cells enter the bursa between 8 and 14 days incubation and have already undergone gene Ig rearrangement, probably in the embryonic spleen and bone marrow, for they express IgM on the surface (see Chapter 4). Within the bursa they undergo rapid rounds of cell division and only within this unique environment does gene conversion occur. In the absence of the bursa an antibody repertoire cannot be generated and a major arm of the immune system becomes non-functional. The chicken antibody repertoire is generated during the late embryonic stage and for a short period after hatching. As the chick ages so its B cells undergo additional rounds of somatic gene conversion and the antibody repertoire becomes expanded until a mature repertoire is achieved around 5–7 weeks when the bursa is fully mature. Thereafter, the bursa begins to regress as sexual maturity approaches and the adult probably relies on post-bursal stem cells in the bone marrow as the source of B cells.

Of course, generating the antibody repertoire in a burst of activity in the young animal has its risks. Any pathogen which targets and destroys bursal cells will have a devastating effect on antibody-dependent immune responses. One such virus is the small RNA virus that causes infectious bursal disease. Infection of the neonate chick with infectious bursal disease virus (IBDV) may cause no clinical disease but destroys bursal B cells leaving the chick incapable of mounting an antibody response to other pathogens, although paradoxically there is a good response to IBDV itself (see Chapter 16). The insidious nature of IBDV leaves the chick vulnerable to opportunistic infections, and unprotected by subsequent vaccinations. So relying on the generation of the antibody repertoire in a single location and over a relatively short-time span is not without its hazards and, perhaps, represents one of the more “risky” strategies birds have adopted.

THE CONTRIBUTION OF THE CHICKEN MHC

Pathogens are diverse, cunning and occupy different niches within the body of the host. Apart from pathogens that are found outside of cells, such as *Clostridium*, *Escherichia coli* and *Bordetella*, there are intracellular pathogens that can be found in the cytoplasm or in cellular vesicles. In addition, retroviruses and herpesviruses integrate into the host's own genome.

To match this diversity, and the different locations where pathogens are found, higher vertebrates have evolved a number of different innate and adaptive immunological mechanisms to improve the chances of survival. Antibodies recognize conformational epitopes on the pathogen's molecules but need to come into direct physical contact in order to neutralize a pathogen, as well as recruit cells and other molecules that bring about disposal. Ig molecules are large and cannot easily enter a viable cell, so an intracellular pathogen cannot be recognized by an Ig molecule unless the pathogen's molecules are expressed on the surface of that infected cells. However, the cellular immune system has evolved a number of methods for recognizing and destroying cells with intracellular pathogens. These mechanisms allow the host's effector lymphocytes to recognize infected, or neoplastic, cells through the expression of proteins that have been proteolysed within the cell and are expressed on the cell surface as peptide fragments. In mammals the molecules that present peptides on the surface of cells are encoded by a highly polymorphic genetic region known as the MHC.

The MHC region was originally recognized through its effects on tissue graft rejection and, of course, GvHR. Two major types, or classes, of MHC molecules are encoded by genes in the mammalian MHC region and expressed on the surface of cells, class I and II MHC molecules (more fully described in Chapter 8). Although both types of molecules are heterodimers: the class I MHC molecule consists of an α -chain encoded by the MHC together with an invariant β_2 -microglobulin molecule from a gene outwith the MHC locus; the class II molecule consists of two peptide chains (α and β) whose genes are found in the MHC region. Unlike the MHC class I heterodimer that is present on most types of cells, expression of class II MHC molecules is restricted to antigen-presenting cells such as macrophages, dendritic and B cells. During synthesis inside the cell both classes of MHC molecules trap peptide fragments in a cleft on, what is to become, the outer surface of the extracellular domain of the MHC heterodimer. On reaching the cell surface these peptides are displayed as peptide:MHC complexes to signal T cells through their T cell receptors. Since even the smallest virus produces a number of proteins, and large pathogens can produce hundreds, there needs to be a large repertoire of T cell receptors expressed by different T cells in order to recognize the multiplicity of peptide fragments derived, not from the host's own cells, but made by pathogens or neoplastic cells. This T cell repertoire is developed within the thymus (described in Chapter 5), where developing T cells with receptors that recognize self-peptides associated with MHC molecules are eliminated, or inactivated, before they mature so as to prevent self-recognition and autoimmunity. Mature T cells capable of recognizing "foreign" peptides are released from the thymus into the periphery and become activated if they recognize peptide fragments expressed on MHC molecules. Interestingly, the T cell repertoire in the chicken is developed in the thymus in a similar way to that of mammals. There is no evidence for a somatic gene conversion mechanism such as that occurs in the avian bursa.

In mammals the MHC is a large and complex region that contains much redundancy (Trowsdale, 1995). In the human, it consists of about 4 million base pairs encoding at least 280 genes. Separate regions contain several MHC class I and II genes (there are a vast number of alleles) that are highly expressed on cells. These regions are separated by third region that encodes immune response genes (class III). Humans express two or three class I molecules and three or four class II molecules that are highly polymorphic. The high polymorphism is probably driven by the ever-changing variations in pathogens (Doherty and Zinkernagel, 1975; Zinkernagel and Doherty, 1979), although different haplotypes appear to confer approximately the same degree of protection against most of the infectious pathogens. In Chapter 8, Kaufman points out that the associations between the human MHC and infectious disease are, actually, very slight.

The chicken MHC is known as the B locus, since it was first identified as a serological blood group locus (Briles *et al.*, 1950) encoding the polymorphic, and highly immunogenic BG antigen. This BG antigen is highly expressed on blood cells and has no known mammalian equivalent. Later it was shown that the B locus must constitute the avian MHC, because of its strong association with cell-mediated immune functions, such as graft rejection, mixed lymphocyte reactions and GvHR. Remarkably, and in marked contrast to the large mammal MHC, the chicken B locus is minute, spanning only 92 kilobases and encoding 19 genes and making it

approximately 20-fold smaller than the human MHC (Kaufman *et al.*, 1999). Only two copies each of class I (BF) and class II β (B/L β) genes are found within the chicken B locus. The marked differences between the chicken MHC and its mammalian counterpart have led Kaufman to argue (see Chapter 8) that the chicken B locus represents a minimal essential MHC that must have evolved after birds and mammals separated some 200 million years ago. Another striking feature of the chicken minimal MHC region is that, not only does it affect a number of important cell-mediated immune functions, but also determines life or death in response to a number of pathogens (Kaufman *et al.*, 1995; Kaufman, 2000). In Chapter 8, Kaufman develops the argument that chickens, with a minimal essential MHC, appear to have adopted a completely different strategy to that of mammals whose MHC is large and complex. The close association between the chicken MHC and disease resistance is fascinating and at first sight seems to be a suicidal strategy. However, many lessons can be learned from studying chicken immunology and its parallel evolution. By studying the minimal chicken MHC, light should be thrown on the important interactions between pathogens and the immune system and the importance of different evolutionary strategies.

THE CONTRIBUTIONS TO VACCINOLOGY

In any review of the “firsts” credited to avian immunology tribute needs to be paid to pioneering developments in the practical uses of immunology; in other words, the use of vaccination. The modern poultry industry relies heavily on vaccines to protect against a wide range of different infectious agents. Vaccinations are frequent and begin from the day of hatching or even before. Chickens are immunized with live-attenuated vaccines and killed vaccines delivered by various routes (injection, aerosol spray, drinking water, etc.) in mass vaccination programmes that dwarf programmes in human medicine.

Every biology student knows that Edward Jenner is the founding father of vaccination. Jenner discovered that cowpox pustules, obtained from an infected milkmaid, protected an 8-year-old boy, James Phipps, against the related smallpox virus. However, further developments in vaccination, and indeed the term vaccination, only came into use about a century later, following studies by one of the greatest nineteenth century scientists, Louis Pasteur. Here again the chicken had a privileged role and serendipity played its part.

In 1878, Pasteur was investigating chicken cholera, a disease with devastating effects, causing chickens to become anorexic, moribund and usually leading to their death. Pasteur investigated the causative agent, now known as the *Pasteurella multocida*, and succeeded in growing the bacteria in culture. The story goes (De Kruif, 1953) that Pasteur’s research was interrupted by a holiday and a culture was left in a flask in the laboratory. Upon resuming his research, Pasteur inoculated chickens with this stale culture. The chickens became sick but then recovered within a few days. We now know that the bacteria had become attenuated and no longer capable of causing mortality. Pasteur did not know this and decided to inject new chickens with a fresh bacterial culture but, unfortunately (or fortunately!), his assistant found that chickens at the local market were in short supply. A number of fresh birds were obtained but those that had recovered from the inoculation with the stale culture needed to be reused. As expected the new chickens all succumbed to the fresh pathogen and died but those that had recovered from the previous treatment with stale inoculum again recovered. Pasteur realized he had achieved with chicken cholera what Jenner had accomplished with smallpox some 100 years earlier, only in this case he had attenuated (weakened) the pathogen by prolonged storage. He called the attenuated culture “vaccine” (Pasteur, 1880) in honour of Edward Jenner and then began a, largely successful, search for similar vaccines against other infectious diseases such as pig erysipelas, sheep anthrax and rabies. The serendipitous discovery of attenuation was another novel finding made using the chicken. Since then the development of vaccines has had far-reaching implications for the health and welfare of both humans and domesticated animals. The search for better, more effective vaccines still goes on apace.

Another first in vaccine development was in the control Marek's disease (MD) a naturally occurring neoplastic disease of chickens. MD became the scourge of the poultry industry in the 1950s and 1960s, causing major problems for animal health and welfare and becoming a huge financial burden. Before the introduction of MD vaccines, morbidity and mortality in laying flocks ranged from 0% to 60% or greater, with losses of 30% being common (Powell, 1986). The development of an MD vaccine represents the first example of widespread use of vaccination to protect against a natural form of cancer (Purchase, 1973). Over the years it has been remarkably effective (Witter, 2001), although not without problems.

MD was first described as a neurological disease (polyneuritis) by Josef Marek (1907). The condition caused paralysis of the wings and legs and was associated with mononuclear infiltrations and enlargement of the major nerves. Later it was observed (Pappenheimer *et al.*, 1926, 1929) that in addition to lesions in the nerves and central nervous system, chickens also developed lymphoid tumours in several visceral tissues (visceral lymphomatosis) such as the ovary, liver, kidneys, adrenal and muscles. With intensification of poultry production the acute form of MD became a dominant feature. Although the introduction of MD vaccines in the 1970s controlled the disease, in some countries problems with vaccine breaks have continued to occur with regularity and there is now good evidence that the causative herpesvirus (Marek's disease virus, MDV) has been able to evade vaccine-induced immune responses by evolving to greater virulence. Since the 1980s, the response of the industry in some countries has been to introduce more aggressive vaccine strategies, using "hotter" vaccines, such as CVI988, either alone or in combination with other MD vaccines (bivalent or trivalent combinations). The most efficacious current MD vaccine CVI988 is derived from a serotype 1 MDV that is weakly oncogenic in genetically susceptible chickens and this has led some to raise the important question (Witter, 1997): Where do we go if hypervirulent MDV pathotypes evolve that can break through the protection of CVI988?

MDV is not the only example of a poultry virus that has changed in response to the introduction of widespread use of vaccines. More virulent isolates of another lymphotropic virus, IBDV were isolated in the late 1980s. IBDV is a small double-stranded RNA virus that encodes only five viral proteins. As already pointed out, IBDV targets B lymphocytes in the bursa of young chicks causing no clinical signs in neonates but causing clinical disease and some mortality in older chicks (see earlier). Chicks are protected by maternal antibodies derived via the yolk, but it became clear in the late 1980s that the very virulent IBDV being isolated from outbreaks was capable of causing disease in the presence of high levels of maternal antibodies. The response of the industry has been to introduce more aggressive ("hotter") vaccines to protect against the more virulent IBDV strains that have evolved under the pressure of vaccine use. The risk, however, is that these hotter vaccines could themselves be capable of causing bursal damage and immunosuppression in chicks that are poorly protected by maternal antibodies or have a susceptible genotype. Here again we have an example of a strategy that is holding at present but may not be sustainable long term. More aggressive vaccines or vaccination regimes cannot be introduced without the risk that the vaccines themselves could be harmful.

These issues have been addressed in epidemiological studies on implications of the use of vaccines on the evolution of pathogen virulence. Researchers (Gandon *et al.*, 2001) were chiefly concerned with the use of different vaccines developed against the malaria parasite and implications for human populations. Mathematical modelling was carried out based on the premise that most vaccines are imperfect and rarely provide full protection from disease. Using various models, these authors predicted that vaccines designed to reduce the growth and/or toxicity of pathogens actually diminished selection pressure against more virulent pathogens. Consequently, subsequent pathogen evolution may lead to higher levels of intrinsic virulence and hence more severe disease in unvaccinated individuals. Such evolution would tend to erode any population-wide benefits, such that overall mortality could increase with the level of vaccination coverage. Interestingly, the authors found evidence of this phenomenon in the practical problems arising from MD vaccination. Current MD vaccines target viral replication and do not prevent infection with MDV, consistent with mathematical modelling that predicts evolution

of pathogen virulence. Yet again, evidence from work on the chicken has proved to be the path-finder and pointed to important problems to take account of in vaccine design.

Embryonic (*In Ovo*) Vaccination

The problems associated with challenge from virulent MDV when chicks are moved into rearing quarters, the vast numbers of chicks requiring vaccinations at the hatchery, as well as the occasional failures caused by manual vaccination, has led to a search for new ways for mass vaccination that can be done at an even earlier stage than the day-old chick. Sharma and colleagues at the East Lansing Regional Poultry Laboratory, USA, demonstrated that chick embryos could be successfully vaccinated against MDV at 17–18 days incubation (Sharma and Burmester, 1982; Sharma and Witter, 1983). The automated INOVOJECT® system, which allows the automated mass application of vaccines to large numbers of eggs (up to 50 000 eggs per hour, Gildersleeve *et al.*, 1993) was developed and has been widely applied in the poultry industries of some countries (for a fuller description, see Chapter 20). In the USA, almost all broilers (approximately 7 billion per year) are vaccinated by this method. *In ovo* vaccination is achieved by puncturing a small hole through the blunt end of the egg with an oblique pointed needle then passing down a smaller needle to deliver a small amount (usually 50 µl) of vaccine into the amniotic cavity. Since the amniotic fluid is imbibed prior to hatching the vaccine is then taken up by the embryo. Interestingly, the reasons why *in ovo* vaccination, applied to such an immunologically immature individual, is so effective remains to be fully explained. Nevertheless, the finding that a higher vertebrate can be protected against challenge early after hatching (birth) by vaccinating the embryo is quite revolutionary and its practical application in mass vaccination a great achievement. The immunological explanations will surely come in due course.

CONCLUSIONS

Compared to mouse and man the avian immune system may seem like a poor relation and yet it has provided important insights into fundamental immunological mechanisms and can claim a number of important “firsts” especially with regard to vaccinology. From the immunological viewpoint, the chicken is, perhaps, the best-studied non-mammalian species. The recent publication of the chicken genome (International Chicken Genome Sequencing Committee, 2004) has provided the opportunity for a “quantum shift” in the search for new chicken genes and exciting possibilities for developing new tools and reagents to study immune responses and immunogenetics. Interest in studying immune responses of other avian species is increasing, including species of wild birds. Ecologists are now taking an interest in measuring immunocompetence and determining its importance as a heritable trait for the survival, both of the individual and the population. Avian immunology is a fascinating and growing field and will surely provide new and exciting insights for mainstream immunology in the future.

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