AN INTRODUCTION TO

Veterinary Immunology

IAN R. TIZARD

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PREFACE

It is hoped that this book will meet the need for a first level textbook of immunology for students of Veterinary Medicine. At the same time it is designed to assist practitioners in analyzing immunological problems by acquainting them with recent developments in the field of immunology as it relates to veterinary medicine.

In general, the reader will find that the text has been written at a relatively elementary level in view of the fact that it is intended for the immunological novice. Because of this the simplification of complex immunological concepts may be offensive to the "immunologically competent" reader; nevertheless I believe it to be justified.

The book itself may be divided into three sections. In the first portion is reviewed the biological basis of the immune responses. This is an area that has expanded dramatically in recent years and that, as a result, has tended to leave those persons not intimately involved in the field floundering in a mass of immunological jargon. The second section of the book is concerned with the role of the immune response in resistance to infectious disease. This area, in which immunology first blossomed, remains the field through which this subject exerts its major influence on applied veterinary medicine. I believe, however, that over the last few years a gap has developed between "basic" and "applied" veterinary immunology, and it is hoped that this section will help to bridge that gap. The final section of the book deals with diseases of immunological origin. This is an area that veterinarians have only recently begun to investigate, and as a consequence, it is difficult to avoid merely listing isolated cases of specific diseases. It is inevitable that our understanding of this area will expand greatly in the next few years.

Many individuals have contributed materially to the development of this book. Among those who offered valuable suggestions and helpful criticism I should particularly like to mention Drs. B. Derbyshire, P. Eyre, A. Fernando, D. G. Ingram, F. Markham, K. Nielsen, F. Rurangirwa, R. Saison, B. Stemshorn, B. N. Wilkie and S. Yamashiro. I am particularly indebted to Dr. D. A. Barnum, not only for reading and constructively criticizing the manuscript, but also for his active encouragement of its production. The illustrations were largely drawn by Ms. Alice Hillock of the Audio-visual Department of the University of Guelph. She is to be commended not only for her artistic skill but also for her patience in dealing with the many requests of the author. I should particularly like to thank Chris Taal and Helen Corbett for typing the

manuscript from my illegible handwriting. I would also like to acknowledge the considerable assistance of the W. B. Saunders Company, especially Mr. Carroll Cann and Mr. Sandy Reinhardt. Finally, but most importantly, this work would never have been completed without the encouragement, assistance and patience of my wife.

If this book serves its function as a useful aid to the teaching of immunology in the veterinary curriculum, then credit must go largely to those mentioned above as well as to that relatively small number of individuals whose research into the immunology of the domestic animals forms the basis for this book. Any faults in this book, however, are mine alone.

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CHAPTER 1

GENERAL FEATURES OF THE IMMUNE RESPONSES

Humoral Immune Response
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If a piece of living tissue is surgically removed from one animal and grafted onto another, it usually survives only for a few days before being "rejected" by the recipient, and consequently dying. This process is significant, not so much for the difficulties it presents to the transplantation surgeon, but because it is a reflection of the capacity of the animal body to recognize and then destroy material regarded as foreign. This process is known as an immune response, and the study of immune responses, their mechanisms and consequences, is known as immunology.

The rejection of foreign tissue grafts is but one form of the immune response. Nevertheless, it is of importance as an indication of the existence of a mechanism whereby cells, differing only slightly from those of a normal recipient, are recognized and promptly eliminated. In general, aged and dying cells are removed from the body by nonimmunological processes. However, cells with minor structural abnormalities may be recognized as "foreign" by the "immune system" and eliminated even though they are otherwise apparently healthy. The immune response to foreign cells may therefore be considered to be an indication of the existence of some form of "surveillance system" which identifies and removes abnormal cells as they arise, thus preventing the occurrence of life-threatening changes such as neoplastic transformation. This concept of surveillance tends to be supported by the observation that individuals whose immune response is suppressed by drugs so that they can tolerate foreign grafts are more liable than normal individuals to develop tumors. The ability to distinguish between normal "self" constituents and foreign material is also essential if the body is to maintain itself relatively free from invasion by microorganisms or parasites. In the absence of an effective immune system, massive infections leading to death are inevitable.

The identification of the defensive functions of the immune system long preceded the suggestion that surveillance for abnormal cells might also be necessary. Thus, in the 11th century the Chinese observed that individuals fortunate enough to recover from smallpox were resistant to further attacks of this disease. Being practical people they introduced the custom of deliberately infecting in-

fants with smallpox in order to protect them from this disease in later life. The great risks inherent in this procedure were acceptable in an era of high infant mortality and on gaining experience with the technique it was found that the least severe reactions were obtained by selecting the smallpox scabs used in this process from the mildest cases available. The technique gradually spread westward to Europe where "variolation," as it was called, came to be widely employed in the latter half of the 18th century. As a consequence of this practice, there occurred a dramatic drop in mortality due to smallpox, resulting in a population explosion that served both to provide soldiers for the Napoleonic wars and to significantly hasten the development of industrial society in Europe.

In 1798, Edward Jenner, an English physician, confirmed the suggestion of one of his patients, a dairy maid, that cowpox could substitute for smallpox in variolation. Since cowpox does not cause severe disease in man, its use effectively reduced the risks incurred in protecting against smallpox to insignificant levels, and the current success of this technique is such that smallpox almost certainly will soon become the first major infectious disease to be eradicated from the world.

The general implications of Jenner's observations were not realized until 1879 when Louis Pasteur in France made some experimental observations on the resistance of chickens to the causal agent of fowl cholera *(Pasteurella multocida)* (Fig. 1-1). Pasteur possessed a culture of this organism which was accidentally allowed to "age" for several weeks on a laboratory bench. Nevertheless, he tried to infect chickens with this culture but without success. Being economically inclined, Pasteur kept these chickens for a second experiment in which they were challenged again, this time with a fresh and virulent culture of *P. multocida*. To his surprise these chickens still did not die. On investigating the problem further, Pasteur realized that this phenomenon was similar in princi-

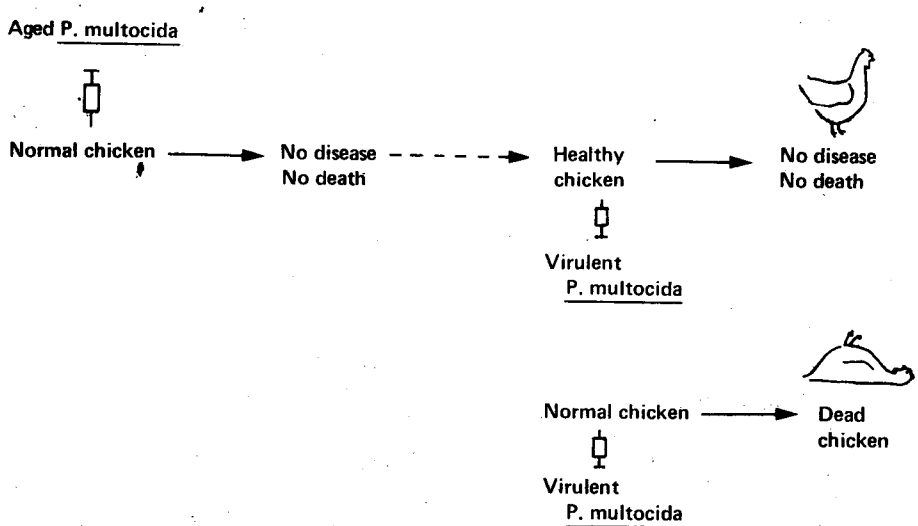

Figure 1-1 Pasteur's fowl cholera experiment.

ple to Jenner's use of cowpox. He therefore called the process vaccination (vacca is Latin for cow). In vaccination, exposure of an animal to a strain of an organism which will not cause disease (an avirulent strain) can provide protection against a subsequent infection by a disease-producing (virulent) strain of the same, or closely related organism. (It is of interest to note at this point that vaccination of poultry against fowl cholera is still relatively unsatisfactory despite Pasteur's initial successes.) Having established the general principle of vaccination, Pasteur applied it to anthrax, rendering the organisms avirulent by growing them at an unusually high temperature. He also developed a successful rabies vaccine by allowing spinal cords taken from rabies-infected rabbits to dry and then using the dried cords as his vaccine material, since the drying process effectively rendered the rabies virus avirulent. While Jenner and Pasteur both used avirulent but living organisms in their vaccines, it was not long before other investigators demonstrated that killed organisms, or even some filtrates taken from bacterial cultures, could also give effective protection in other diseases.

HUMORAL IMMUNE RESPONSE

After Pasteur had discovered that it was possible to produce resistance to infectious agents by vaccination, it was soon recognized that the substances which provided this resistance could be found in blood serum. (Serum is the clear yellow fluid which is expressed when freshly drawn blood has clotted and the clot has been allowed to contract.) It was shown, for instance, that serum from a vaccinated animal could be used to transfer immunity to a nonvaccinated one. For example, if serum is obtained from a horse made resistant to tetanus toxin by vaccination and this serum is injected into a normal horse in appropriate quantities, then that normal horse will become temporarily resistant to tetanus. The serum from the immune horse is known as tetanus antitoxin and is widely used for the prevention of this disease.

The factors found in serum which confer resistance in this way are known as antibodies. Antibodies to tetanus toxin are not found in normal horse serum but are produced as a result of vaccination. Tetanus toxin is just one example of a foreign substance which stimulates antibody production. The general term for such a substance is "antigen." If an antigen is injected into an animal, then antibodies are produced which can combine with that antigen. Antibodies usually only combine specifically with the antigen which stimulates their production so that, for example, the antibodies produced by exposure to tetanus toxin react only with tetanus toxin. If serum containing these antibodies is mixed in a test tube with a solution of tetanus toxin, then a visible precipitate develops as a result of the combination of antibody with the toxin. In addition, the antibody serves to "neutralize" the toxin so that it is no longer toxic. It is by means of this neutralization process that antibodies can protect animals against the lethal effects of tetanus toxin.

It is possible to follow the time course of the immune response of a horse to tetanus toxin by bleeding it at intervals after vaccination (Fig. 1–2). The amount of antibody in the serum may be estimated either by measuring the amount of precipitate formed on adding toxin, or alternatively, by measuring the ability of

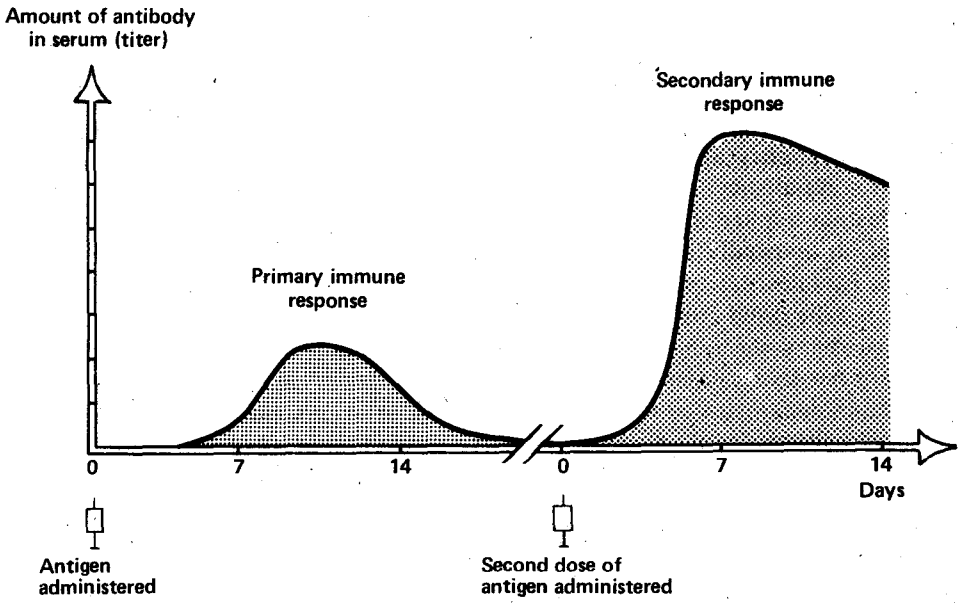

Figure 1–2 The time course of the immune response to an antigen as measured by serum antibody levels.

the serum to neutralize a fixed amount of toxin. Both methods yield approximately the same results. Following a single injection of toxin (or its chemically neutralized derivative, tetanus toxoid) into a horse which has never been exposed to it previously, no response is detectable for several days. This is known as the lag period. Antibodies become detectable about one week after the first injection and the amount present in serum then climbs to reach its highest level by ten to fourteen days before declining rapidly. In general, the amount of antibody formed, and hence the amount of protection conferred, during this first or "primary" response is relatively small. If, some time after the first, a second dose of toxin is given to the same horse and the antibody response again followed, then the lag period tends to last for no more than two or three days. The amount of detectable antibody then rises rapidly to a high level before commencing to decline slowly. A third dose of toxin when given to the same animal results in an immune response characterized by an even shorter lag period and a still higher and more prolonged antibody response. The stimulation of resistance to disease through the use of multiple injections of antigen in this way forms the basis of current vaccination techniques employed against infectious diseases.

As we have seen, the response of an animal to a second dose of antigen is very different from the first in that it occurs much more quickly and antibodies reach very much higher levels. This "secondary" response is specific in that it can be induced only by an antigen identical to the first. A secondary response may be invoked many months or years after the first injection of antigen although its magnitude does tend to decline as time passes. A secondary response can also be induced even though the response of the animal to the first injection of antigen was so weak as to be undetectable. Thus, the antibody response is characterized by possessing the ability to remember previous exposure to an an-

tigen. For this reason, the secondary immune response is sometimes known as an anamnestic response (anamnesko is Greek for recollection).

If a second dose of antigen is given to an animal still possessing serum antibodies remaining after its primary immune response, then the level of these antibodies may drop for a few days before the secondary immune response gets under way. This so-called "negative phase" occurs as a result of the injected antigen binding and removing antibodies from the circulation. It should also be noted that multiple injections of antigen do not lead to greater and greater immune responses indefinitely. The total level of antibodies in serum is relatively well controlled, so that they tend to plateau at a constant level even after multiple doses of antigen or exposure to many different antigens.

CELL-MEDIATED IMMUNE RESPONSE

If a skin graft is transplanted from one dog to a second unrelated dog, it will survive for about ten days; the graft will initially appear to be healthy, and vascular connections will be established between the graft and its host. By about one week, however, these new blood vessels will begin to degenerate, as a consequence of which the blood supply to the graft will be cut off so that it will die and be shed. This slow rejection process is known as a "first-set reaction" (Fig. 1–3). If a second graft is taken from the original donor and placed on the same recipient, then that second graft will survive no longer than one or two days before being rejected. This rapid rejection process is known as a "second-set reaction." Thus, in the process of graft rejection we see that the response to a first graft is relatively weak and slow and analogous to the primary antibody response, while a second graft stimulates a very rapid and powerful second-set reaction similar in many ways to the secondary antibody response. Graft rejection, like antibody formation, is specific in that a second-set reaction, in general,

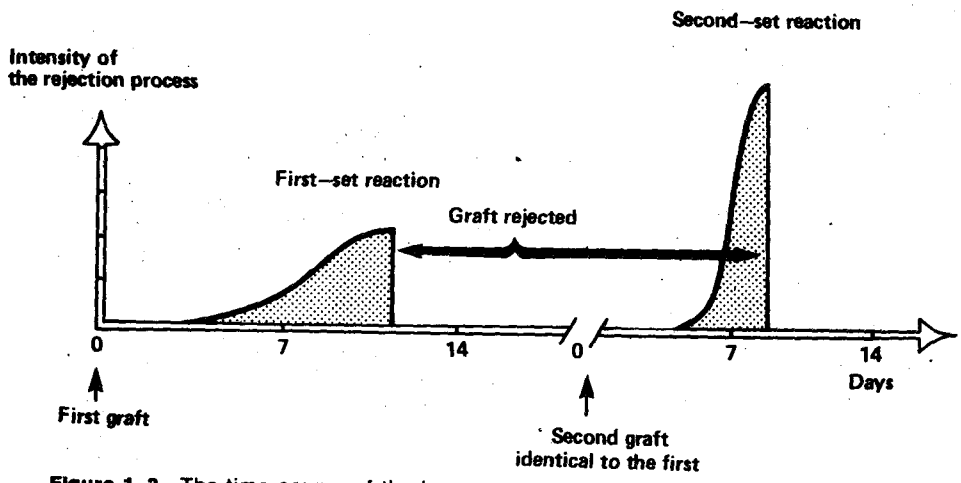

Figure 1–3 The time course of the immune response to a foreign skin graft. Notice how similar this diagram is to Fig. 1–2.

occurs only if the second graft is from the same donor as the first. Like antibody formation, the graft rejection process also possesses a "memory," since a second graft may be rapidly rejected many months or years after loss of the first.

However, the graft rejection process is not entirely identical to the process involved in protection against tetanus toxin, since it cannot be transferred from a sensitized to a normal animal by means of serum antibodies. The ability to mount a second-set reaction to a graft can only be transferred between animals by means of living cells. The cells which can perform this function are known as lymphocytes and are usually derived from the spleen, lymph nodes or peripheral blood. Because of this, we must conclude that the process of graft rejection is mediated primarily by lymphocytes and not by serum antibodies.

TOLERANCE

We have already discussed how it is essential for the immune system to be able to identify antigens, such as tetanus toxin, or grafts from other animals as foreign. It is an absolute corollary to this that the immune system must be able to recognize antigens on its own cells as being "not-foreign," and it must not mount an immune response against them. In other words, the immune system must be "tolerant" to self-antigens. If this tolerance breaks down, then disease will occur (autoimmune disease) as either antibodies or lymphocytes attempt to eliminate the offending antigen. Tolerance, which occurs in both the cell-mediated and antibody-mediated immune systems, can be considered to be but another form of a normal immune response. For example, tolerance, as shown by an inability to react to a specific antigen, may be induced by an appropriately administered dose of that antigen. Tolerance is quite specific for the inducing antigen and, like other forms of the immune response, may be boosted only by re-exposure to that same antigen. If not re-exposed to that antigen, then tolerance is gradually lost.

The immune responses may therefore be considered to consist of three general types. They include the antibody and cell-mediated immune responses and tolerance. In considering the necessity for an immune system, it was pointed out that there is a requirement for self-surveillance as well as one for resistance to invasive microorganisms. It is tempting to suggest that the cell-mediated immune responses are a reflection of this surveillance function while the antibody-mediated responses reflect the protective function. Such a distinction is not absolute, however, since antibodies may contribute to graft rejection, and cell-mediated immune responses can participate in resistance to many infectious diseases. Tolerance, on the other hand, represents an essential protective mechanism which serves to prevent an animal from being damaged by an indiscriminate immune response.

MECHANISM OF THE IMMUNE RESPONSES

In some ways the immune system may be compared to a totalitarian state in which foreigners are expelled, citizens who behave themselves are tolerated, but

those who "deviate" are eliminated. While this analogy must not be pursued too far, it is readily apparent that such regimes possess a number of characteristic features. These include border defenses and a police force which keeps the population under surveillance and promptly eliminates dissidents. Organizations of this type also tend to develop a pass system so that foreigners not possessing such identifying features are rapidly detected and dealt with.

Similarly, when antigen enters the body it first must be trapped in such a way that it can be recognized as being foreign. If so recognized, then this information must be conveyed either to the antibody-forming system or to the cell-mediated immune system. These systems must then respond promptly by the production of specific antibody and/or cells which are capable of eliminating the antigen. The immune systems must also store the "memory" of this event so that on subsequent exposure to the same antigen, their response will be considerably more efficient. In our totalitarian state analogy, the information would be filed away for future use.

We can therefore consider the basic requirements of the immune systems to include a method of trapping and processing antigen (Fig. 1–4); a mechanism for reacting specifically to the antigen or, in other words, an antigen-sensitive cell; cells to produce antibodies or to participate in the cell-mediated immune responses; cells to retain the memory of the event and to react specifically to

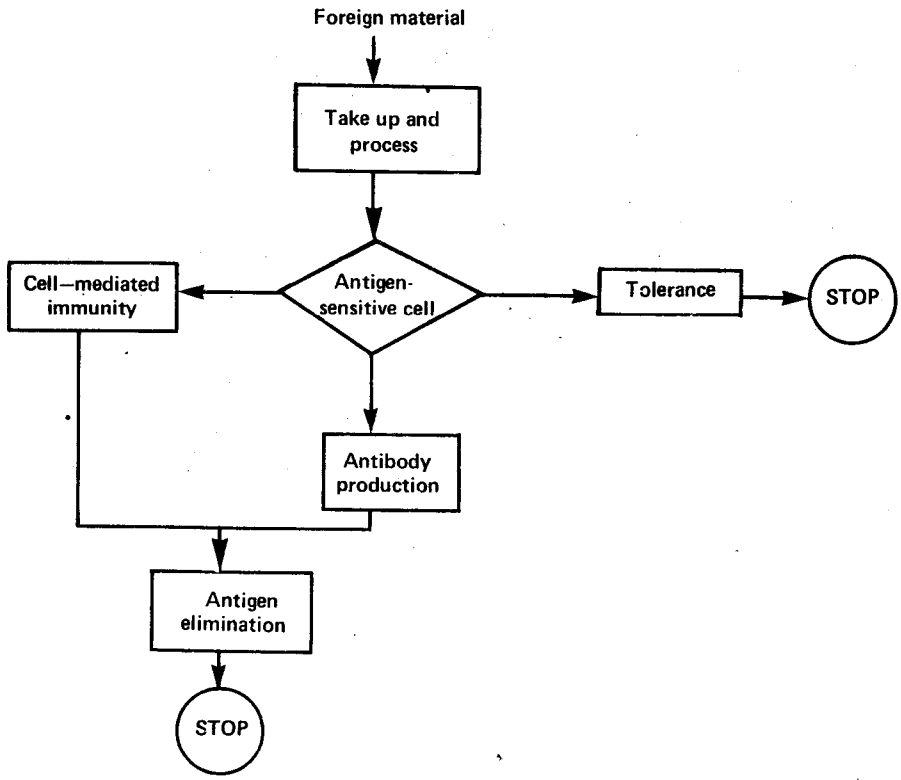

Figure 1–4 A simple flow diagram showing the essential features of the immune responses.

the antigen in future encounters; and finally cells to eliminate antigen. All of these cell types are recognized within the body. Antigen is trapped, processed and eventually eliminated by cells known as macrophages. Antigen-sensitive cells, both those present at the beginning of a primary response and those memory cells which initiate a secondary response, as well as the effector cells of the cell-mediated response, are identified as small lymphocytes, while antibody-producing cells are derived from lymphocytes and are known as plasma cells.

In subsequent chapters we will examine each of these basic requirements of the immune responses in turn.

SELECTED SOURCES OF ADDITIONAL INFORMATION

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Many Immunology Journals contain occasional articles of Veterinary interest. Some of the most important of these include: Cellular Immunology, Clinical and Experimental Immunology, European Journal of Immunology, Infection and Immunity, International Archives of Allergy and Applied Immunology, Immunology, Journal of Experimental Medicine, and Journal of Immunology.

Veterinary Immunology

Reviews

Although the literature on veterinary immunology *per se* is scant, the following symposia organized by the American Veterinary Medical Association provide an excellent source of information not only on immunity to diseases, but also on the immune systems in the domestic animals.