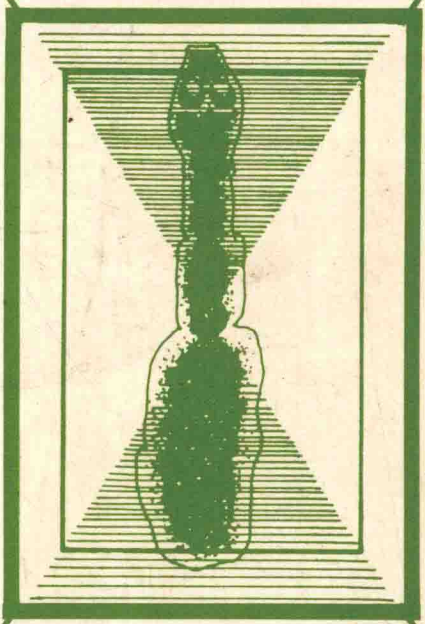
# THE IMMUNOLOGY OF PARASITIC INFECTIONS

A Handbook for Physicians, Veterinarians, and Biologists



By
Omar O. Barriga

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Veterinarians, and Biologists

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# The Immunology of Parasitic Infections

# Preface

The intelligent man must often ask himself why, in the dawn of the 21st century, there are still 800 million of his fellow men infected with hookworms, 300 million with amebiasis, and 200 million with schistosomiasis; why 400 million people are at risk of contracting malaria and 50 to 150 million actually acquire it every year; why 10 million human beings harbor the agent of Chagas' disease and 35 million are under its menace; why livestock is so scarce and horses nonexistent in vast regions of Africa covered by luxurious vegetation; why the United States, where the control of parasitic diseases has reached a high degree of efficiency, lost 1.2 billion dollars per year in the decade 1951–1960 as a result of animal parasitism.

The possible answers are numerous, but the basic reason may reside in the fact that animal parasites are much older than mankind itself: the parasitic protonematodes originated with the insects, the protocestodes with the crustacea, and the digenetic prototrematodes with the mollusks. The parasitic protozoa are probably much older than the parasitic helminths.

Throughout more than 600 million years, and accompanying the vertebrates since their phylogenetic origins, the parasitic species had the time and opportunity to genetically select and to adapt to the peculiarities of their hosts in a very effective manner. We do not know the number of species (undoubtedly high) that succumbed in this process, but those that survived must have taken advantage of every biological "loophole" that facilitated their continuity.

Nature, free of our anthropocentric bias, for millenia took proper care that the parasitic as well as the host species had a fair opportunity to persist in time. The relatively scarce pathogenicity of contemporary animal parasites and the comparative inefficiency of the defense mechanisms of the hosts are eloquent proofs of the perfection of the host-parasite relationship as we know it nowadays. It is not surprising, then, that the efforts of the professionals of human or animal health have had meager results in their attempts to destroy such an old association that has already come successfully through so many vicissitudes.

The advent of the lymphoid system in the vertebrates and its further refinement in the homoiotherms must have represented a formi-

dable obstacle that relatively few parasitic species were able to conquer. Nevertheless, it is astounding that those species are able to coexist with a physiological mechanism such as the immune system, the primordial purpose of which, if we yield to a pinch of teleology, is precisely to rid the organism from exogenous or endogenous invaders. This coincidence seemed so unnatural that only 50 years ago, as many years after the celebrated experiments of Pasteur in Pouilly-le-Fort, Hegner directed the attention of the scientific community to the fact that the host-parasite association in blood protozoa was similar to that existing in bacterial infections. Until fairly recently, even individuals with training in biomedical sciences believed that the immune responses to animal parasites should be peculiar to these organisms and different from the reactions to other pathogenic agents.

The first attempt to present the immunity against parasitic animals in a comprehensive manner was the monumental work by Taliaferro, published in 1929. Twelve years later, Culbertson repeated the feat by reviewing the advances achieved in that period in a text that even today can be read with benefit. The prodigious advancement of immunology in the last decades (the number of papers in immunology rose from 220 in 1940 to 7660 in 1970) and the increasing amount of investigations related to the immunity against animal parasites allowed almost 30 years to elapse before a team of 35 authors dared gather the information on the discipline in two comprehensive volumes (Jackson, Herman, and Singer, 1969–1970). More recent works that attempted to give detailed and erudite accounts of the state of the art have been edited by Soulsby (1972) and by Cohen and Sadun (1976), with the collaboration of numerous specialists.

Although the fine details of the immunology to parasites have gone far beyond the possibilities of exhaustive examination by nonspecialized readers, the study of the immune response against animal parasites is becoming more and more important for the professionals connected with human and animal health, and for biologists. Problems such as the natural refractoriness to malaria in some human populations, the mechanisms responsible for the lethal anaphylaxis in the terminal phases of piroplasmosis, the differences in the evolution of infections by leishmanias, the significance of the EVI antibodies in Chagas' disease, the precise identification of hydatid disease and visceral larva migrans, the correct interpretation of the presence of specific agglutinins in bovine trichomoniasis, new possibilities for the prevention of sequelae in schistosomiasis, and the effective prophylaxis of respiratory nematodiases have already been or are in the process of being solved by immunological studies.

In addition, parasite immunology is contributing considerably to revealing the phylogenetic relationships among diverse parasitic organisms and is constantly discovering new facets of the fascinating host-parasite association. Finally, in recent years, parasites have been demonstrated to constitute quite adequate probes for exploring diverse peculiarities of the immune system.

Thus the time has come to write a text that explains the basic principles and the practical implications of the immunity against animal parasites, for physicians, veterinarians, biologists, and the students of these disciplines who do not have the time, the vocation, or the need to pursue advanced studies of the subject. This book is directed to them and also, as an introduction to the specialty, to the parasitologists and immunologists who have not had training in the complementary discipline. I think that even some colleagues may welcome the identification of some of the numerous areas that are in most urgent need of research. The book places emphasis on the parasitic infections occurring in the Americas. I hope that the necessary lack of depth in a text of this nature is duly compensated by a coordinate treatment that emphasizes principles and applications rather than fine details.

For the benefit of those readers who need to refresh their knowledge of immunology, two chapters have been specially included: one that summarizes the most important concepts in the discipline, and another that briefly explains the molecular mechanisms of the immunological tests commonly used in clinical parasitology. For those who do not remember the parasitological aspects, the most relevant characteristics of the natural history of the parasites that are not transmitted directly have been mentioned at the beginning of each chapter. Those readers who may desire to deepen their knowledge in any specific field should find abundant opportunity to do so in the bibliographic references at the end of each chapter. Necessarily, a large proportion of these are review papers. Nevertheless, they cite the original publications on which the opinions expressed in the text are based.

Finally, perhaps in no field of human activity is it as true as in the academic life that "no man is an island." My own training has had the benefit of the contribution of too many people to name here. It is only fair, however, to mention Professor Amador Neghme, of the University of Chile, who initiated me in the fascinating task of teaching and doing research in the biomedical sciences and encouraged my interest in the humanities; Drs. Norman D. Levine and Diego R. Segre, of the University of Illinois, who were patent examples of superior scholarship and unequaled kindness; and Dr. E. J. L. Soulsby, citizen of the world, and lately at the University of Cambridge, with whom I had the honor

to work for five unforgettable years in Philadelphia. My gratitude goes to all of them. Anything good in this book is a reflection of their teachings and examples; anything incorrect is my exclusive responsibility.

Omar O. Barriga

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To
Inés,
Omar Jr.,
and Alvaro,
for their
love and
support.

# The Immunology of Parasitic Infections

# Contents

Chapter 1

# Review of Basic Concepts of Immunology

The advent of the vertebrates in the parade of evolution brought into existence a group of animals far more complex than earth had seen before; prolificacy alone was no longer able to secure their continuity in time. The coordination and preservation of these novel organisms demanded systems much more sophisticated than those previously existing; some were acquired by perfecting old physiological mechanisms and a few were virtually newly created. The immune system was among the latter.

The primordial function of the immune system is the preservation of the biochemical identity of the individual, by detecting and reacting against exogenous or endogenous invaders (pathogens and tumors, respectively). In the classic writings, immunity has often been described as homologous to defense. Even accepting that the general evolutionary "purpose" of immunity must have been to provide the vertebrates with an efficient mechanism to fight disease, the immune reactions must be recognized as automatic responses that occur whenever the required conditions happen, regardless of their end result. Thus, in real life, we find that some immune responses are deleterious for the invader, others are injurious for the host, and, finally, others are indifferent for either partner.

This chapter discusses briefly the main features of the immune system in order to form a basis for understanding its participation in the production, course, and control of parasitic infections, as well as its possible use for practical applications. There is currently a high degree of interest in immunology, and the field is undergoing explosive growth; therefore a large number of textbooks on the subject has been written in the last few years. Most of them are quite adequate, so that selecting references for further study becomes almost a matter of personal preference. The interested reader may benefit from consulting the book by Benacerraf and Unanue (1979), which provides an excel-

lent introduction to general immunology. The textbook edited by Fudenberg et al. (1978) is a more comprehensive work that presents up-to-date information on the basic and clinical immunology of humans. The manuals by Tizzard (1977) and the Olsen and Krakowka (1979) are particularly appropriate for those interested in the immunology of domestic animals.

## STRUCTURE AND GENERAL FUNCTION OF THE IMMUNE SYSTEM

The structural basis of the immune system is the lymphohematopoietic tissue, and the main reactive cell is the lymphocyte. A general view of the immune system indicates that it was acquired only by the vertebrates and that, among them, it evolved to reach the highest complexity in the birds and, particularly, in the mammals.

The structures that form the immune system are generally described at three levels: level 1, the stem cell compartment (fundamentally the bone marrow), where new lymphocytes are produced; level 2, the central or primary lymphoid organs (thymus and bursa), where the bone marrow lymphocytes acquire their capacity to respond to immunological stimulation; and level 3, the peripheral or secondary lymphoid organs (e.g., spleen, lymph nodes, Peyer's patches), where the lymphocytes are stimulated and respond to their corresponding antigens.

The lymphocytes that acquire immune competence in the thymus are known as thymus dependent or thymus derived, or, simply, *T lymphocytes* or *T cells*. Once differentiated in the thymus, they migrate to the peripheral lymphoid tissue and become particularly abundant in the blood, lymph, and paracortical zones of the lymph nodes. The lymphocytes that mature in the bursa of Fabricius are called bursa dependent or bursa derived, or *B lymphocytes* or *B cells*, and are found principally in the bone marrow and in the germinal centers of the lymph nodes. The human spleen contains approximately equal proportions of T and B cells. The anatomical equivalent of the bursa has not been identified in mammals, but there is evidence that the bone marrow exerts bursa-equivalent functions in these vertebrates.

The recognition of a substance as foreign or "nonself" by the immune system is limited to macromolecules that have a certain degree of complexity (*antigens*). Practically all foreign proteins with a molecular weight of 10,000 or more, numerous "nonself" polysaccharides of 60,000 or more, and the combinations of extraneous proteins with polysaccharides are effective antigens. A considerable number of substances of low molecular weight (*haptens*) are also able to stimulate an immune response when they are conjugated with an antigenic pro-

tein. Oligosaccharides, lipids, and nucleic acids, as well as synthetic compounds, occasionally behave as haptens.

In practically all cases, the recognition of the antigenic substance, either free or constituting part of a more complex structure (cell, virus), begins with its ingestion and "processing" by macrophages. There is no consensus yet on what happens to the antigen during this processing, but apparently those portions of the antigenic molecule that will be recognized as foreign (*antigenic determinants*) are distributed on the surface of the macrophage and become easily accessible to the lymphocytes.

The next step in the chain of the immune response is still obscure and has been the subject of numerous studies and interpretations. Simply explained, the native antigen is able to prime the effector lymphocytes genetically predetermined to react with it, but this stimulation is not enough to initiate their multiplication. The macrophage-processed antigen, however, triggers a subpopulation of T lymphocytes called *regulatory cells*; at least functionally, these are divided into a "promoter" or "helper" set and an "inhibitory" or "suppressor" set. The helper cells appear within the first hours of the immune response and produce soluble substances that stimulate the proliferation of the effector lymphocytes already primed by the antigen. The suppressor cells become functional 3 or 4 days after the initiation of the immune response and set a limit to the multiplication of the effector lymphocytes, which, otherwise, would continue multiplying like a neoplasia.

Following the sequential stimulation by the native antigen and by the product of the helper cells, the effector B lymphocytes begin to divide in rapid succession for several generations until they differentiate to plasma cells, which are active producers of antibody. During this proliferation, some daughter cells remain at the stage of small lymphocytes as "memory cells." They may persist for years in the body and, when stimulated by a subsequent dose of the same original antigen, they initiate a more rapid, intense, and effective immunological response (*secondary* or *anamnestic* response) than occurred on the first contact with the antigen (*primary* response). A few antigens constituted by numerous repeated monomeric units (such as the polysaccharides of pneumococcus or the lipopolysaccharides of enterobacteria) are able to stimulate B lymphocytes without the assistance of helper cells; the antibodies produced in these cases are virtually pure immunoglobulin M. This observation may be particularly relevant to parasitology, since helminths contain abundant polysaccharides.

It is not clear at the moment whether effector T lymphocytes are as dependent on the activity of helper cells to proliferate as are the B lymphocytes. At any rate, subsequent to the introduction of the antigen

in the host, the effector T lymphocytes divide for several generations until becoming mature cells. When these cells enter in contact again with the same antigen that stimulated their formation (either because it remained in the body or it was acquired de novo), they release a number of humoral substances collectively known as *lymphokines*.

Certain materials called *mitogens* are able to stimulate the proliferation of T cells, B cells, or both in the absence of antigen or of helper cells. Several of them are proteins extracted from plants, but substances with mitogenic activity have been recently reported to have been found in some parasites. At least in theory, these substances might be able to enhance or abolish the response to a particular antigen, by expanding or preempting the population of lymphocytes predetermined to react with it.

The immune response can be artificially enhanced by the administration of compounds generically called *adjuvants*, together with the antigen. One of the most widely used is Freund's adjuvant, which contains mineral oil and an emulsifier to assure its intimate mixture with the antigen. Dead *Mycobacterium* may or may not be added (complete or incomplete adjuvant, respectively). Because of the severe local inflammation produced by Freund's adjuvant, other adjuvants (e.g., aluminum hydroxide) are preferred for clinical applications. Adjuvants not only increase the immune responses to an antigen, but can also favor the production of one particular manifestation of immunity over the others: Freund's complete adjuvant facilitates the expression of cell-mediated immunity; *Bordetella pertussis* and *Ascaris* extracts stimulate the formation of immunoglobulin E antibodies; bacterial endotoxins enhance antibody production. Also, substances that normally do not produce a detectable immune response can be made antigenic by inoculating them along with potent adjuvants.

The development of the lymphoid system occurs early in ontogeny: small lymphocytes appear in the peripheral blood of human fetuses at 7–8 weeks of gestation and their lymph nodes are populated already from the fourth month on. However, the ability to respond to different antigens (which is genetically predetermined for each lymphocyte) is attained at various times around birth: lambs born with congenital toxoplasmosis present specific antibodies produced prenatally, whereas lambs infected with *Haemonchus contortus* do not develop protective immunity if they are under 4–6 months of age. Before acquiring immune competence, lymphocytes are particularly sensitive to very large or very small doses of antigens: experimentally, it has been verified that administration of antigens in these ranges often induces lack of immunological responses to subsequent conventional doses of the same antigen (*immunological tolerance*). This inhibition of the responses

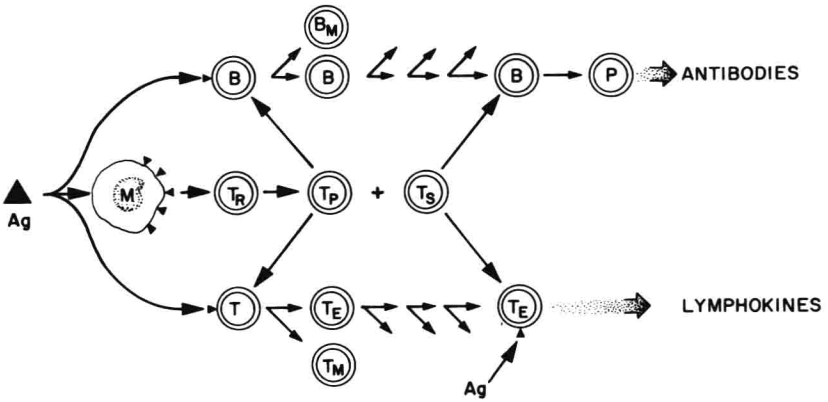


Figure 1. Schematic representation of the cellular events entailed in the immune response. The newly acquired antigen (▲) primes competent B and T lymphocytes (**B** and **T**) and is ingested and processed by appropriate macrophages (**M**). The macrophage-processed antigen subsequently stimulates two subpopulations of regulator T cells ($\mathbf{T_R}$): promoter T lymphocytes ($\mathbf{T_P}$), which induce the multiplication of the immunocompetent cells beginning a few hours after the introduction of the antigen; and suppressor T lymphocytes ($\mathbf{T_S}$), which inhibit their proliferation starting on the third or fourth day. The stimulated B lymphocytes divide for several generations, generating memory B cells ($\mathbf{B_M}$), and finally mature to plasma cells (**P**) that are active producers of antibodies. The stimulated T lymphocytes also divide several times, generating memory T cells ($\mathbf{T_M}$) and effector T cells ($\mathbf{T_E}$); these latter will produce lymphokines on reencounter with the homologous antigen.

usually passes after a few weeks, but it may persist indefinitely if the presence of the antigen continues. Conventional amounts of antigen, on the other hand, may accelerate the acquisition of immune competence for the homologous antigens. Although there is little solid evidence yet, some observations suggest that these phenomena of tolerance or rapid maturation might occur in the course of parasitic infections in very young hosts.

From the account above (summarized in Figure 1), it is clear that immunity can be expressed in two general ways: by formation of antibodies, or by production of lymphokines. Traditionally, these two branches of immunity have been called humoral and cell-mediated immunity, respectively.

## HUMORAL IMMUNITY

Antibodies are glycoproteins that constitute the group known as immunoglobulins (Ig). Two major characteristics define the immunoglobulins: (1) chemically, they possess a structure with two heavy and two light polypeptide chains, arranged in a typical fashion (see below); and