The Immune System and Infectious Diseases

Editors:

Erwin Neter, Buffalo, N. Y.

Felix Milgrom, Buffalo, N. Y.

4th International Convocation on Immunology

1974

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Introduction

Since its inception, the Center for Immunology has sponsored a series of biennial convocations with the aim of bringing together scientists from all over the world to present and discuss the current status and future trends in various areas of immunology.

The present volume contains the articles based on the presentations given during the four-day conference held June 3–6, 1974, on the subject 'The Immune System and Infectious Diseases'. It is this area of investigation which is the cradle of immunology and which was initiated more than a century ago. The extraordinary advances made recently in the various areas of immunobiology and immunochemistry significantly affect our understanding of immunologic processes operating in infectious diseases regarding pathogenesis, recovery, and prevention. Many of these modern trends are the subjects of the presentations of scientists from Belgium, Canada, Great Britain, Israel, Sweden, the USA, and West Germany.

Six major topics were selected as the themes of this volume.

'The Maturation of the Immune System' focuses on recent advances made in our understanding of the immune response of the fetus, newborn animal and infant, and the clinical implications of the findings to pathogenesis and diagnosis.

'Microbial Agents as Immunosuppressants and Adjuvants' have attracted renewed attention, particularly because of their effects on viral infections and malignancies.

The study of 'Host-Parasite Relations with Particular Reference to Cross-Reacting Antigens' has opened new vistas to our understanding of the development of immunity to certain infections because of previous contact with unrelated microorganisms. These studies are leading to novel immunization procedures and serodiagnostic approaches.

'The Local Immune Response' has been re-investigated intensively in recent years, particularly since the role of secretory antibodies in immunity has been clearly established. The role of local cellular immunity only recently has become the subject of investigation.

A survey of 'The Role of RNA of Microorganisms in Immunity to Infection' was considered timely because of the impact of these studies on vaccine development.

As the final topic of the Convocation, 'The Humoral Immune Response and Aspecific Factors' was selected in order to focus on factors other than antigens, antibodies, and immunocytes which affect immunologic reactions.

The sessions were concluded with critical reviews of the topics or the implications and applications of recent observations.

The Ernest Witebsky Memorial Lecture on the subject of 'Symbiosis in Immunology and Medicine' was presented by LEWIS THOMAS. This contribution is included in the present volume and will be of unusual interest to all immunologists.

To all who contributed to the success of the Convocation and the preparation of the present volume, sincere thanks are expressed, particularly to the authors, the members of the Program Committee, numerous members of the Center for Immunology and the Department of Microbiology, and, especially to Mrs. ETHEL LEE HELFFENSTEIN, who functioned so efficiently as Managing Editor. We are also grateful for financial support through grants from many organizations. The cooperation of S. KARGER AG, the publisher of this volume and the three preceding volumes, is much appreciated.

January 1975

ERWIN NETER, Buffalo, N.Y.
FELIX MILGROM, Buffalo, N.Y.

Contents

The Role of RNA of Microorganisms in Immunity to Infection

The Humoral Immune Response and Aspecific Factors

Ernest Witebsky Memorial Lecture

The Immune System and Infectious Diseases. 4th Int. Convoc. Immunol., Buffalo, N.Y., 1974, pp. 2–11 (Karger, Basel 1975)

Symbiosis as an Immunologic Problem

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It is obvious that when symbiosis is working well, regardless of how intimate the association may be between pairs or groups of organisms, it is not an immunological problem at all, and that is in itself a problem.

One part of the puzzle is this: how was it possible for the intricate, complex, specific and highly destructive system which we recognize as immunology to have evolved in the face of such extensive, powerful and equally specific systems designed to maintain separate organisms in such close associations that they often appear to have become one organism? Immunologic mechanisms seen designed to maintain and preserve the absolute identity and separateness of organisms, to keep things apart, utilizing an elaborate apparatus dependent upon specific chemical communication. Symbiotic relationships, on the other hand, are maintained by equally specific chemical communication systems, to accomplish what seems a fundamentally opposed objective, incompatible with immunology.

A central problem for the success of both kinds of enterprise concerns the chemical labeling of self. In immunological circles, we tend to think of this as a two-sided adversary problem, involving the chemical distinction between self and non-self; the arrangement is set up so that the individual organism can protect itself and its various parts while engaged in defense against all others. In symbiosis, the self-recognition mechanisms are necessary for another, quite different but equally urgent reason, having to do with the need to recognize one's partner and to be recognized back, in order to join up.

Is it possible that the self-recognition mechanisms involved in symbiotic relationships are in some way related to those with which we work in immunology? On the surface, this would seem quite unlikely; neither antibodies nor lymphocytes seem to have any role to play in symbiotic associations. Instead, quite different sorts of chemical signals, some of them certainly pheromones, appear to be utilized for the specific communications used in symbiosis. Some of the most elaborate and complicated symbiotic systems involve marine invertebrates, unendowed with any recognizable kind of immunologic equipment. Moreover, symbiotic arrangements evolved very early in the life of the earth, probably representing the dominant mode of life for many millions of years before the emergence of vertebrates.

However, and perhaps for this very reason, it may be useful to consider the possibility that there is, in fact, a relationship in evolutionary terms. It is conceivable that today's system for the immunologic sensing of a vertebrate organism's internal environment may have evolved from earlier, more primitive mechanisms designed for interdependence among different organisms.

There are, to begin with, very good reasons to believe that symbiosis was, for a very long time, not only a more or less universal process in nature but also a fundamental driving force in evolution itself. This must have begun at around the time when prokaryotic life had already acquired the capacity for photosynthesis, and a sufficient concentration of oxygen had been elaborated into the earth's atmosphere to make oxidative energy systems both possible and necessary for the continuing development of life. It is not known, of course, how we evolved from non-nucleated microbial cells to eukaryotic cells, but one of the more plausible explanations for this step is that certain cells joined together to form larger, more elaborate cell masses, and cell nuclei evolved within the new aggregates. TIMOURIAN [13] and others have proposed that multicellular organisms must have evolved by a similar process of joining together; cells with the features of today's protozoa became permanently associated in cell masses, perhaps even underwent cell fusion, and the progeny of such associations displayed the beginnings of cell differentiation within the earliest metazoan organisms by utilizing one or another of their properties as a specialty – phagocytosis, for example, or ciliated movement.

This is, of course, a highly speculative matter, but there is one well-established fact, derived from recent advances in cell biology, which indicates that some such process of joining up among different cells must have occurred very early in the evolution of nucleated cells. The mitochondria of all eukaryotic cells, and also the chloroplasts and other plastids of plants, are now believed to be essentially foreign symbionts, derived originally from ATP-forming bacteria in the case of mitochondria, and from blue-green algae in the case of chloroplasts. The evidence for this is solid enough to make it highly probable, which is perhaps as far as it can be taken, considering that the theory is, by its nature, beyond proving to the point of certainty. The semi-autonomy of mitochondria, and their resemblance to bacteria, are based on the knowledge that these organelles have their own DNA, RNA and ribosomes, each differing from that of the host animal cell and each very similar to bacterial DNA, RNA and ribosomes; the mitochondrial membranes, with which the DNA is associated as in prokaryotic cells, are structurally and biochemically more like bacterial membranes than those of eukaryocytes; the protein-synthesizing enzyme systems of mitochondria are, in part at least, closely similar to those of bacteria. A similar case is made for the origin of chloroplasts from photosynthesizing prokaryotes. It is possible, but not yet proved, that other cytoplasmic entities in nucleated cells will also turn out to be endosymbionts, derived from the microbes of a billion years or more ago.

It is of incidental interest that the origin and nature of mitochondria and chloroplasts were firmly predicted with the cytological techniques of the late 19th century, by SCHIMPER [11] for chloroplasts in 1883, and by ALTMANN [1] for mitochondria in 1890. There are few examples of such accurate guesswork to match these in the whole history of biology. Now, almost a century later, with these long shots confirmed, I wonder if we have even begun to take in the implications of these astonishing facts.

That symbiosis is a nearly universal way of life is most conspicuously evident in the life of the sea, perhaps because of the relative antiquity of so much of this life. There are no genuinely solitary, single, independent organisms. Every form is dependent upon other forms; creatures live inside each other, occupy the same burrows and carapaces, share food, live off each other, carry each other around, even clean each other. It is not a random arrangement in any sense. All of the partners are perfectly specific for each other, and, in most cases, the partnerships involve the exchange of equally specific chemical signals, received and read by specific chemoreceptors. Some of the negotiations are conducted by olfaction, some by taste, others by obscure sorts of molecular recognition at membrane surfaces. Anemones and damsel fish become associated by virtue of the capacity of the fish to produce, adaptively, a surface material which the anemone seems to recognize as self, so that the fish are not stung by the tentacles. Hermit crabs carry their own particular species of anemone on their shell surface, to which the anemone attaches itself by recognition of a specific shell surface protein. Polychaete scale worms live in permanent symbiosis with starfish and other echinoderms, with specific attractants which each echinoderm species produces to localize the polychaete at the host's surface. The barnacles that attach themselves to whales are specialized for this particular species, and are not encountered elsewhere.

Symbiotic arrangements are probably no less common among terrestrial forms of life, but they are less conspicuous. The most spectacular example of total, perfect symbiosis that I have heard of is the protozoon, *Myxotricha paradoxa*, which lives exclusively in the intestinal tract of an Australian species of termite. This organism, like other intestinal protozoa in termites, is an indispensable symbiont, responsible for the digestion of the wood fragments on which the termite depends for its living. It is a conventional partnership, but this particular protozoon is itself not a single creature but a symbiotic arrangement. Under electron microscopy, the ciliary structures at the *Myxotricha* surface turn out to be spirochaetes attached at regular sites along the surface membrane. Moreover, there are oval structures embedded near the base of each spirochaete, and these have turned out to be bacteria, probably responsible for some of the digestive enzymes which digest the termite's wood. This animal, or collection, is a living diagram to illustrate the evolution of motile protozoa, but it also alludes to the method by which nucleated cells themselves may have been pieced together by ancient, symbiotic aggregates of smaller forms of life. Parenthetically, I can think of a lot of problems in biomedical science that are considerably less important than this protozoon, and I wish someone would think of a way to fit this creature into the National Cancer Program Plan.

There are numerous instances of bacterial symbionts occupying crucial roles in the economy of plants, insects and vertebrates. The bacterial colonies termed mycetocytes, which live in the form of small, essential organs in the tissues of certain insects, are especially intriguing; they seem as indispensable for the insect as rhizobial bacteria are for leguminous plants, but nothing is known of their function beyond its indispensability. Bacterial symbiosis with other bacterial populations is one of the most generalized of biological phenomena; many bacterial species in the soil of the earth (some microbiologists have suggested most) are unable to live without the contributions of other microbes with which they coexist.

Even viruses know symbiosis. Mutually dependent, paired viruses are recognized among bacteriophages as well as among animal viruses. Some viruses behave like symbionts within their own hosts; the bacteriophages of diphtheria and hemolytic streptococci, after establishing lysogeny, enable the host cells to produce their exotoxins; the code for toxin is information belonging to the virus. Unless infected by virus, the diphtheria bacillus is not a pathogen.

Symbiosis is one of the great general properties of biology. In a certain sense it is the most fundamental of all the ones we know about, since it is, in an extended form, the mechanism that holds the entire ecosystem of the earth together. WHITAKER and FEENY [15] have proposed the global term 'allelochemics' for the enormous chemical communication network by which all the collective varieties of life keep in touch with each other, modulating each other's growth, regulating space allocations, and distributing resources around with equity. Some of the signals are information about territorial limits, food supplies, air quality, moisture, dryness, soil properties and other environmental news, but the most important of them are surely the declarations of self. The system cannot work at any scale, from the tiniest ecological niche to the Sargasso Sea, to the whole earth, without certainty concerning this specific piece of information for each creature.

It is astonishing how firmly the pronouncement of selfness is made by organisms with no sort of immunologic equipment whatever. Annelid worms are reported to reject homografts while accepting autografts. The primitive branched coelenterate, *Gorgonacia*, will fuse with parts of its own organism but will reject explants of tissue from other, neighboring Gorgonians. This is done non-immunologically, and, according to THEODOR [12], by a mechanism which would be considered quite novel in immunologic circles. THEODOR [12] placed explants of various sizes, from different Gorgonians, in close apposition with each other, and observed that the smaller of the pairs always underwent dissolution and vanished. He then found that the process was not due to any discernible aggressive action on the part of the larger explant; on the contrary, the melting away of the smaller fragment was a form of suicide. When the small bit was treated with actinomycin-D or puromycin, no dissolution occurred; when the large bit was similarly treated, the small one still vanished. The dissolution was evidently due to the signal received by the smaller piece that there was a larger one nearby and it was now time to bow out, to retire.

How is the sense of self conveyed in nature? So far, we know for sure of only two clear-cut mechanisms, although there are undoubtedly more still to be learned about. The first, of which we know a fair amount in some detail, is the immunologic recognition system, for which the best working models are the H-2 and HL-A histocompatibility antigens of mice and man. The second is the system for olfactory recognition of self and non-self, mediated by pheromones,

In the immunologic model, the histocompatibility antigens are the self-markers, displayed at the surfaces of all cells excepting perhaps the trophoblast and some neoplastic cells. The immunologic response which a mouse makes to self-marking antigens from a different mouse species is mediated by T lymphocytes, utilizing surface receptors whose nature remains quite unknown. Here, then, the signal for self is the H-2 antigen complex, and the receptor is a T lymphocyte. While the details of the effector mechanism remain on the unclear side, the mechanism is itself exquisitely selective, precisely specific and potentially destructive. In this way, homografts of tissue from one mouse are destroyed when placed in the tissues of a mouse of another line.

For the olfactory mechanism, there is abundant evidence that animals of various species rely on smell for the accurate identification of their own and other species, and, also, even more accurately, for the identification of sex partners. The problem of self-marking in the individual sense – that is, the marking of a single animal to distinguish it from all others of its kind – has been less extensively studied, but there can be no doubt that such mechanisms do exist. It is possible that they may exist generally, but the technical problems involved in proving this are obviously formidable and perhaps impossible. However, there are three experimental models, involving fish, man and mice, in which satisfactory evidence of individual self-marking has been obtained.

The first models involve minnows and bullheads. The earliest demonstration of individual recognition by pheromones was made in 1941, by Göz [4], using minnows. Much later, studies by Todd [14] with the yellow bullhead, an extraordinarily intelligent and perceptive fish, have established the matter beyond question. Not only were bullheads found to recognize each other as individuals, by olfactory signals alone, but they also showed the ability to recognize changes in the social standing of individual fish as the outcome of disputes over territory or leadership. When the leading bullhead lost his position, his friends knew of the change even before he did, by the change in his smell.

It is not known whether humans have any mechanism quite like this, but it is at least certain that each of us is marked by his own specific, self-identifying odorant, or medley of odorants, even though we do not ordinarily have any consciousness of this ourselves. The evidence comes from experiments with dogs. It has been known for centuries that trained hounds can track a man across open country by his scent, and distinguish his particular scent from that of all other men. Recently, laboratory studies of the phenomenon have confirmed the fact, although the source and chemical nature of the odorant remains to be determined. Dogs can detect the odor of a light fingerprint on a glass slide, and can distinguish this fingerprint from all others over a period as long as 6 weeks, when the scent finally fades away [6]. The most conclusive evidence comes from tracking experiments with identical twins, in whom it has been shown that the odors of the twins are also identical [5].

The actual nature of human self-marking odorants remains unknown, but it must be a substance, or a group of substances, for which the dog's olfactory receptors have a fantastic discriminatory perception. Since it appears to exist on everything we touch, it would seem most likely to be a product of skin secretion, but this tells us almost nothing. If it is anything like the known pheromones of other species it is likely to be a relatively small molecule, but it would either have to have a sufficiently large and plastic molecular structure, or else be a collection of different molecules which could be displayed in varying combinations and concentrations, in order to account for the enormous number of varying signals required for the labeling of each human as self. It is obviously an extremely complicated problem, but no more difficult than the analogous problem for immunologic self-labeling. Indeed, one wonders whether the same tracking hound might not be put to work on both. Is it possible that HL-A antigen has an odor all its own?

The evidence for individual self-marking by pheromones in mice is less conclusive, and is largely based on experiments with mating behavior, where the chief concern has been with sexual cues rather than self-marking. Nevertheless, the results suggest that self-marking does exist, and is individual-specific. WHITTEN [16] has shown that inbred mice do not exhibit the so-called 'Bruce effect', which is the blocking of pregnancy caused by reappearance of estrus when a newly mated female is exposed to pheromones from another male, different from the original sire. Within inbred lines, the 'strangeness' of a second male cannot be sensed. It is obviously more complicated than the other models for self-recognition, since it involves pheromones produced by androgens as well as individual markers, and the effector response, i.e., pregnancy-block, is undoubtedly due in part to the sex odorant; very likely, a medley of pheromones is involved. Nevertheless, the model seems to be a workable one. Cross-breeding experiments, on a limited scale, have already been reported, and it seems a certainty that the individual specific pheromones of inbred mice are genetically determined.

The matter is raised here in order to make the point on which I wish to conclude. If, as I suggest, there is a fundamental relationship of some kind between the immunologic recognition system and the more ancient mechanisms for partnership identification required for symbiotic arrangements, the mouse model offers a possible experimental approach to the matter. It is known from the findings of BENACERRAF and McDEVITT [3] that the genes of