somal RNA template, which determines sequence of amino acids and coiling and folding of the polypeptide chain.

The function of the antigen has then to be sought elsewhere. Several possibilities come to mind, how antigen might interfere without imposing structural specification.

(1) Antigen could act by removing an inhibitor (natural antibody?) from the ribosomal template, thus permitting the assembly of RNA-amino acid complexes on the template to form new antibody. This it can only do in cells, potentially capable of synthesizing this antibody and carrying it naturally.

(2) Antigen could react with cell surface antibody (especially in secondary response) and intracellular antibody to produce cytotoxic complexes, perhaps with complement fixation, which could stimulate cell metabolism and eventually result in cellular proliferation and differentiation (see also [9,12,13]).

(3) Antigen might act either as such or in the form of e. g. peptide or carbohydrate fragments as the source of specific nutrition for the various kinds of cells producing antibody and stimulate their growth and thus give rise concomittantly to production of antibodies in these stimulated cells.

No information is available yet on any of thsee points, but they are liable to experimental testing.

Zusammenfassung

Einige Probleme der Struktur und Biosynthese der Antikörper werden auf der Basis neuerer Ergebnisse und Theorien diskutiert. Unter den vielen ungelösten Problemen werden hervorgehoben:

1. Die Frage nach der Aminosäuresequenz des «aktiven Zentrums» von Antikörpern.
2. Der biochemische Einfluss des Antigens auf die Zelldifferenzierung und die Faltung der Polypeptidketten.
3. Die Notwendigkeit der zellfreien Antikörpersynthese.
4. Die definitive *de novo* «Induktion» von Antikörper-Produktion *in vitro*.
5. Ein Entscheid über die Fähigkeit individueller Zellen, in ihrer Antikörpersynthese mono- oder multispezifisch zu reagieren.

How Specific is Immunity?

By Hubert Bloch*

The term, 'specificity', is derived from *species*. It characterizes properties as pertaining to a species, restricting them therefore to one kind. For instance, Diphtheria toxin is specific for the microorganism, *C. diphtheriae* since it is not known to occur in the cells of any other species. Likewise, the antibodies produced in an animal after the injection of diphtheria toxin must also be specific. They combine selectively with diphtheria toxin. As a consequence of this reaction, the toxin loses its toxicity. *In vivo*, the specific binding capacity of the antitoxic antibody results in protection against the effects of the toxin. It is by this mechanism that acquired immunity functions in diphtheria.

In connection with the study of infectious diseases, specificity has become an important element in our thinking. Pathogenic microorganisms are usually thought of as 'specific' agents causing 'specific' disease syndromes. In turn, under the impact of an infection, the diseased organism mobilizes 'specific' defense mechanisms resulting in 'specific' immunity to subsequent attacks. In a few diseases (of which diphtheria is an example), the chemical agent mediating the disease symptoms is known to be a toxin which can be isolated and purified. There is usually little or no chemical difference between the toxins obtained from various strains of toxin producers of the same species of microorganisms. The same antibody will therefore neutralize all of these products.

In most infectious diseases, however, the pathogenic effects cannot be attributed to a known toxin. Yet, infection may still result in specific immunity. Such is the case in measles or chicken pox. The pathogenic viruses being of a single stable type, immunity is acquired against this one type (and therefore specific). But examples of this sort are rare. In other virus diseases, e. g. influenza, identical clinical symptoms are caused by a great variety of viral agents which differ in their serologic types and leave little or no cross-immunity against infections with other types of the same virus. True, with respect to any one particular virus strain, the immune response as such is equally specific in these cases as it is with measles, but with regard to the manifestations of the disease caused by these viruses, there is very little specificity. Similar pathological symptoms are brought about by serologically different virus strains and the immunity resulting from infection with one type does not necessarily confer protection against subsequent attacks from viruses of other types.

There is among these viruses a high degree of variability. Mutants appear at a frequent rate and the serologic types prevailing in successive epidemics are sufficiently different from each other so as to provide no

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immunologic protection outside their own type. This situation is by no means typical of virus infections alone. Bacterial diseases vary in the same manner from infections with genetically stable strains resulting in specific acquired immunity (e. g. anthrax) to disease entities caused by strains with a high mutation rate leaving little protection against subsequent infections with serologic variants (e.g. Salmonellae). Thus, in regard to protection against subsequent infections, the effectiveness of the specific immune response hinges on the serologic stability of the infectious agents and becomes limited with increasing bacterial or viral variability.

In contrast to this situation, there are many infections where specific immunity seems to play a minor part in the overall defense picture. The infectious agents are not known to cause the formation of protective antibodies. Nevertheless, some secondary resistance seems to be acquired in the course of an infection. In these instances, the mechanisms mobilized by the host are termed non-specific, because their identity appears to be independent of the infectious agent eliciting their formation.

This fact by itself would not suffice to deprive such mechanisms of their specificity label. Common antigens may quite conceivably exist amongst a number of otherwise unrelated infectious agents. Although their occurrence in more than one species would make such antigens 'nonspecific', the immune response they elicit is nevertheless 'specific', though for the antigen rather than for the organisms in which it occurs. Cross-immunity between unrelated microorganisms has long been known to exist, as for instance, between typhus rickettsiae and Proteus X 19.

The question therefore arises: Is all so-called non-specific immunity based on the existence antibodies elicited by widely distributed antigens? Or is there significant non-specific resistance, the mechanism of which cannot be traced to antigen-antibody reactions?

The present discussion is limited to *acquired* resistance. We are not considering differences in susceptibility to infections among various species. As pointed out repeatedly by Doerr, lack of susceptibility to infection is not be confounded with resistance. There are only very few susceptible host species for any given potential parasite and a search for the mechanisms permitting the establishment of a host-parasite relationship would be more fruitful than to look for defense mechanisms preventing successfull infection. The case becomes different, however, where a species is potentially susceptible. Here, special defense mechanisms may prevent an infection from establishing itself and a number of such mechanisms seem to lie outside, or at the periphery of the realm of classical immunity. Phagocytosis and bactericidy of body fluids, e.g. play important parts in the defense against infections and their relationship to acquired immune mechanisms must be considered. It is common knowledge that antibodies (opsonins) enhance the phagocytic activity of the reticulo-endothelial system. This enhancing effect, however, is immunologically specific.

Other observations are more difficult to interpret. Thus, it has been known for over half a century that the serum of many animal species possesses remarkable antibacterial and antiviral activity[1]. However, there seems to be no relationship between the susceptibility of a species to certain infections and the bactericidal activity of this species' serum against the infectious agents in question, nor does previous exposure to such agents seem to have anything to do with this serum activity.

The serum factors responsible for this activity are called 'natural' antibodies. They are γ-globulins, indistinguishable physico-chemically from immune γ-globulins. Wilson and Miles summarize the problem as to the origin of natural antibodies as follows: Natural antibodies may owe their origin to any one of four mechanisms: actual infection with the corresponding microbe; infection with another microorganism, sharing a common antigen; the entrance via the intestinal tract, or any other route, of antigenic material capable of stimulating the production of cross-reacting antibodies; or the formation of such antibodies as a by-product in the normal functioning of the antibody-forming apparatus altogether apart from any specific external stimulus[2].

The first of the four mechanisms is possible wherever inapparent infections cannot be excluded, i.e. where the antibody-forming species is a potential host for the microbe in question and where ecologic conditions render infection possible. The antibodies elicited in this fashion would then be regular immune antibodies rather than 'natural' antibodies.

The second and the third of the four postulated mechanisms may well operate in more instances than is generally believed to be the case. As mentioned above, it is not unfrequent for microorganisms to share common antigens. If the antibodies which these antigens elicit are functional in conferring anti-infectious immunity, their role must be considered. Inapparent infections are certainly more frequent than is generally believed, and the ingestion of bacterial components is a daily event in anyone's life.

The answer as to just how significant the two possible mechanisms are must come from observations on germ-free animals. Animals reared in a germ-free environment should not have natural antibodies if the second of the four mechanisms is responsible for their occurrence. On the other hand, even in a sterile environment the third mechanism could be effective since

[1] E. Bürgi, Arch. Hyg. (D) *62*, 239 (1907).

[2] G. S. Wilson and A. A. Miles, in Topley and Wilson's *Principles of Bacteriology and Immunity*, Fourth edition (Williams and Wilkins, Baltimore 1955).

dead bacteria or non-bacterial material sharing common antigens with microorganisms may still be ingested and elicit the formation of specific or cross-reacting antibodies. Thus, germ-free rats and chickens reared in a sterile environment were found to have antibodies (agglutinins) against staphylococci[3]. There was suggestive evidence that the food of these animals was contaminated with the corresponding cocci before it was autoclaved and that the ingestion of dead organisms led to the formation of antibodies. Similarly, evidence has been presented that the antihuman blood group B agglutinins which are regularly found in the serum of chicks are elicited by absorption from the intestinal tract of a strain of *E. coli* which possesses high blood group B antigenicity and is a frequent contaminant of the natural habitat of chicks found in their normal intestinal flora[4,5].

These examples to which others could be added clearly demonstrate that infection with, or ingestion of homologous or heterologous antigens can be responsible for the appearance of so-called natural antibodies; that these antibodies are in no way different from immune antibodies; and that they are specific with regard to the antigen which elicited their formation. In case these antibodies happen to fit certain bacterial or viral antigens, they would have the same function in resistance against infection as homologous immune γ-globulins. Depending on whether they were elicited by homologous bacterial antigens or by cross-reacting heterologous materials, their effect would have to be called specific or non-specific.

The fourth of the possible mechanisms, i.e. the formation of natural antibodies apart from any specific external stimulus thus remains the only one which would clearly distinguish natural antibodies from immune γ-globulins. Students of immunology have always been impressed with the high degree of specificity which characterizes the immune response. Indeed, serologic methods are among our finest tools for the differentiation of closely related, chemically similar macromolecular products such as proteins or polysaccharides. On the other hand, one might be equally impressed by the *limits* of specificity of the immunologic reaction. The classical work of LANDSTEINER[6] cites numerous examples of cross-reactions between an antibody and entire families of antigens, or, *vice versa*, between an antigen and families of antibodies. Indeed, there are few antibodies which do not to some extent cross-react with chemically similar antigens. Specificity expresses itself merely quantitatively, the titer against the specific antigen being higher than against the others. If there is any merit in the fourth of the proposed mechanisms, γ-globulins must be shown to occur in the total absence of all possible antigenic stimuli.

As is well known, the γ-globulin level in the serum of newborn humans or animals is low, but tends to rise within a few weeks after birth. Comparative experiments with normal stock and with germ-free animals indicate that this rise is due to exterior antigenic stimulation[7]. Despite the fact, however, that within a few weeks after birth, normal stock animals have significantly higher γ-globulin levels than are found in germ-free animals, it is important to stress in this connection that some γ-globulin is always found, even in the apparent absence of all antigenic stimulation. Could such γ-globulins then be regarded as true natural antibodies? Unfortunately, too little is known as yet about the ability of these γ-globulins to interact with other macromolecules. To obtain this information, one would have to react serum from germ-free animals with a great number of randomly selected potential antigens and test these sera for antibacterial activity, etc.

Antigen-antibody reactions follow the laws of protein-protein interactions. To illustrate the specificity of protein interactions, EMIL FISHER used the lock-and-key symbol. This view was adapted by EHRLICH in explaining his sidechain theory of immune reactions and antibody formation. One of the main objections to accepting EHRLICH's theory was the unlikelihood that the countless variety of antibodies (locks) really existed which was needed to satisfy the specificities of all conceivable antigen configurations (keys). This objection, however, need not be valid. In a recent article on antibody formation, TALMAGE[8] uses again the same parabolic language when he points out that to any size set of locks and keys, there can be made a masterkey, similar but slightly different from all individual keys, yet opening all the locks. According to this view, it is not necessary to postulate the existence of an unlimited number of pre-formed antibodies, each one fitting just one of all conceivable structures of antigens. Rather, a very limited number of pre-formed antibodies would suffice to serve as templates in the final production of specific antibodies. The fit of the relatively few pre-existing globulins would not be perfect, but like the masterkey, it would be adequate to function until the accelerated formation of more specific, better fitting antibodies has been initiated.

In summary then, it seems that a great majority of the so-called non-specific or natural antibodies are in fact elicited by *bona fide* antigens and that such activities as bactericidy of serum are either due to specific reactions with the eliciting antigen itself, or to cross-reactions with other microorganisms sharing a common molecular configuration with the original antigen. Only

[3] M. WAGNER, Ann. N. Y. Acad. Sci. *78*, 261 (1959).

[4] G. F. SPRINGER, R. E. HORTON, and M. FORBES, J. exp. Med. *110*, 221 (1959).

[5] It is interesting to note that even such eminently 'natural' antibodies as red blood cell agglutinins may often be acquired rather than endogenous.

[6] K. LANDSTEINER, *The Specificity of Serological Reactions* (Harvard University Press, Cambridge 1945).

[7] B. S. WOSTMANN, Ann. N. Y. Acad. Sci. *78*, 254 (1959).

[8] D. W. TALMAGE, Science *129*, 1643 (1959).

those γ-globulins which were formed in the absence of any antigenic stimulus, i.e. the non-maternal γ-globulins occurring in newborns or in germ-free animals may then be regarded as truly natural non-specific antibodies.

In recent years, there has been a renewed interest in other non-specific defense mechanisms, especially after PILLEMER *et al.*[9] described a bactericidal serum factor which they called properdin and which they thought had many of the properties claimed to be essential characteristics of an important non-specific defense mechanism: It had a broad spectrum of activity, it was stimulated by such non-specific and non-antigenic agents as bacterial endotoxins and was thought to be independent in its action of the presence of humoral or cellular antibodies. It was a protein but differed from antibodies in many respects. A great number of studies, too numerous to be reviewed here, have been undertaken to test the validity of these claims. There seems to be fair agreement on the observed facts, but there is disagreement on their interpretation. Especially, one crucial question remains open: Are not many of the effects which were attributed to the properdin system actually caused by properdin *and* antibody; and is not the contribution of properdin rather secondary in importance? Convincing evidence has recently been published showing that a careful new evaluation is needed and that properdin seems indeed to play only a minor role in the so-called non-specific defenses of the host as measured by the bactericidal properties of serum[10,11].

We could dismiss the question as a mere semantic problem and satisfy ourselves with the fact that there are serum factors, whether called natural antibodies or properdin, which have a major function in defense. However, the question which was asked in the title of this essay still remains to be answered. How specific is immunity? Wherever acquired immunity against infection is attributable to circulating serum constituents, it seems that in the great majority of cases the activity rests with antibodies. These are either homologous, i. e. elicited by the infectious agent with which they react, or heterologous for having been brought about by materials which were different, but structurally related to the infectious agent. In regard to the infectious agent, the homologous antibodies are specific, the heterologous nonspecific. However, both types of antibodies are acquired by the same immunologic mechanisms and function alike. The same is presumably true for normal γ-globulins occurring in the serum without any external stimulus. Where antibodies are concerned in acquired resistance, the difference between specific and non-specific immunity rests solely in the nature of the antigenic stimulus which elicited antibody formation. Operationally, specific and non-specific immunity are similar. There is an impressive body of evidence showing that antibodies are indeed responsible for most manifestations of acquired resistance against infection and that the role of other serum factors is far from clear at the present time.

Zusammenfassung

Der vorstehende Artikel enthält eine allgemeine Diskussion über die Grenzen der Spezifität, die der erworbenen Resistenz gegen Infektionskrankheiten zugrunde liegt. Es wird die These vertreten, dass spezifische Mechanismen von grösserer Bedeutung sind als häufig angenommen wird und dass sog. unspezifische Abwehrkräfte in vielen Fällen sich nicht von echten Antikörpern unterscheiden lassen.

[9] L. PILLEMER, Ann. N. Y. Acad. Sci. *66*, 233 (1956).
[10] R. A. NELSON, JR., J. exp. Med. *108*, 515 (1958).
[11] E. OSAWA and L. H. MUSCHEL, J. Immunol. *84*, 203 (1960).

On the Role of Non-Specific Factors in the Pathogenesis of Infectious Disease

By E. SUTER*

After the first discoveries of bacteria as agents of disease the search for pathogenic components produced by these agents resulted in significant findings. Consequently, the ability to produce toxin or toxin-like substances by pathogenic microorganism was assumed to be the major contributory factor in the elicitation of disease. This assumption provided a satisfactory explanation of the pathogenesis of disease in which isolated and purified toxins were shown to reproduce the clinical picture of the disease, as in diphtheria, tetanus, gas gangrene, and others. However, the limitations of this concept were realized when it became apparent that microorganisms, obviously capable of causing severe and often fatal infections, failed to yield products with any striking pathogenic effect. In infections caused by such agents no substances were found which could elicite in a susceptible host a clinical picture similar to the one produced by the living pathogen. Therefore, other mechanisms in addition to those based on toxin production were considered. Disturbances of the normal functioning of the host's tissues were recognized to be caused by the metabolic activity of the pathogen. Furthermore, the interaction of antibodies or antibody-like substances with bacterial components were found to cause severe tissue damage. At least some of the

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