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Immunopathological Mechanisms in Bacterial-Host Interactions [and Discussion]

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Immunopathological mechanisms in bacterial–host interactions

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The ability of bacteria to cause immunopathological damage in the host may take a variety of forms. These pathways may be conveniently grouped under three major headings: (1) organisms that can cause damage via shared antigenic determinants between host and bacterium; (2) those organisms that suppress the host's response; and (3) organisms that release substances with specific biological properties or have receptors for specific tissue sites.

The group A streptococcus is among the most versatile of these bacteria because it appears that it may use all three pathways in various streptococcal-related disease states. In rheumatic fever and chorea it appears that cross-reactive antigens play a major role in inducing immunopathological damage in that there is both a heightened humoral and cellular reaction by the host to these cross-reactive determinants. Recent evidence also indicates that rheumatic fever individuals express certain B cell antigens that may be associated with susceptibility to the disease.

In the other complications of streptococcal infections, namely poststreptococcal glomerulonephritis, the bacterium uses both suppression of the host's immune response and the excretion of a particular protein common to all nephritis-associated strains to achieve its immunopathological damage. In this context, other examples of bacterial–host interactions will be discussed as evidence for the common pathways used by microbes to cause immunopathological damage in the host.

Introduction

Although at first glance the task of covering the field of immunopathology of bacterial infections may seem to be almost insurmountable, a closer inspection suggests that many bacteria use a number of similar pathological pathways to achieve their goals. This is not, of course, to belittle in any manner the subtle mechanisms and subterfuges that each bacterial species has developed to gain entry into the host and establish itself as a pathological entity. One need only mention the streptococcal M protein fimbria which in some unknown fashion permits the organism to avoid phagocytosis; the pili of the gonococcus, which is apparently used to attach itself to the mucosal wall; the direct action of diptheria toxin on enzyme systems of the cell; or the seemingly endless ability of some bacteria to mutate their surface proteins to avoid the host's immune system, as but a few samples of the extraordinary diversity of the bacterial world.

In many of these instances the result is a direct invasion of the host and unless checked by the host's defences or some exogenous help such as antibiotics, the result is death. The above approach is self-destructive and does not permit a continued symbiosis of bacterium and host. To avoid this situation, many bacteria have developed a more indirect approach to establishing a pathological foothold that is not self-destructive and permits the continual growth and dissemination of the organism. It is this area of immunopathology on which I shall concentrate: it represents pathways of pathological damage that many bacteria share in common. These pathways may be conveniently grouped into three major headings:

(1) organisms that exert their effect by microbial mimicry;
(2) organisms that establish pathological footholds by suppression of the immune response;
(3) organisms that release substances with specific biological properties leading to immunopathological damage.

Among the many microbial examples one has to choose from, the group A streptococcus is high on the list since it employs under different conditions all three forms of microbial–host interactions. I shall therefore confine most of my comments to this particular microbial–host interaction but shall draw from other examples to illustrate the universality of these immunopathological pathways.

TABLE 1. A SELECTION OF CROSS-REACTIONS BETWEEN MICROBES AND MAMMALIAN TISSUES

organism	tissues	possible disease association	references
Streptococcus pyogenes	heart, brain, kidney, etc.	rheumatic fever	Zabriskie *et al.* (1970)
Streptococcus mutans	heart	?	van de Rijn *et al.* (1976)
Klebsiella	HLA-BW27 lymphocytes, man	ankylosing spondylitis	Ebringer (1981), Seager *et al.* (1979)
Salmonella	mouse tissues	*Salmonella* infection	Rawley & Jenkin (1962)
Pneumococci, Gram-negative bacteria	blood-group substances	? susceptibility to infection	Finland & Curren (1940), Springer *et al.* (1961)
E. coli	colon tissue	ulcerative colitis	Perlman *et al.* (1965)

BIOLOGICAL MIMICRY

The term molecular mimicry was originally coined by Damian (1964) in his studies on parasitic organisms, but it is now clear that the sharing of antigenic determinants between host and microbe is a quite common event and probably occurs far more frequently than the evidence has so far indicated. Table 1 is a partial listing of the known cross-reactions between bacteria and various tissue determinants. It is my belief that these cross-reactions are occurring constantly and, as we shall see later, it is perhaps only in the genetically programmed individual that immunologically relevant pathological damage occurs.

To concentrate on only one organism, the group A streptococcus is capable of mimicking certain antigenic determinants of almost every major organ of the body (Zabriskie 1982). However, in the context of this paper I shall concentrate on only two major cross-reactions in detail, namely antigens cross-reactive with muscle, including cardiac muscle, and those reactive with brain antigens.

Without boring the reader with the details of how these cross-reactive antigens were discovered and elucidated, it will suffice to say that Kaplan's investigations (Kaplan & Frengley 1969) and our own work (Zabriskie & Freimer 1966) led to the conclusion that certain streptococcal antigens shared antigenic determinants with cardiac antigens. Rabbits immunized with whole streptococci or isolated cellular components of them produced an antibody that bound by immunofluorescence to human heart sections. A similar antibody was found in the sera of patients with acute rheumatic fever, and the staining pattern of these two antibodies appeared to be identical. The localization of the streptococcal antigens in question has been a controversial subject, but it now appears that there are two antigens: one that resides in the cell membrane and one closely associated with the M protein moiety.

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Examination of a large number of sera from patients with recent streptococcal infections and their sequelae revealed heart-staining antibody in most of them during the acute streptococcal infection and emphasizes the point that most individuals do develop heart reactive antibodies after a streptococcal infection. However, the amount of antibody detected in rheumatic fever individuals at the onset of their disease was strikingly different from that found in patients with uncomplicated streptococcal infections. Two to three weeks after uncomplicated streptococcal infection, sera from the latter had little or no heart-reactive antibody, whereas sera from patients with acute rheumatic fever had antibodies detectable at a tenfold dilution. The concept that these antibodies are intimately associated with the disease process has been strengthened by our serial studies of recurrences in rheumatic fever patients. In almost every patient studied, the antibody appears with the initial attack and has either declined or disappeared before the second attack. In some instances the antibody is still present in the serum of the patient and is boosted with the second attack. In others, a series of intercurrent streptococcal infections is needed to 'prime' the individual, and with these infections heart reactive antibody reappears in lower titres in the sera of these individuals. The final infection leading to the disease process is associated with a return of the high titres of heart-reactive antibody.

These observations of long intervals between attacks, with the appearance of heart-reactive antibodies in the serum before recurrence, makes it tempting to speculate that repeated streptococcal infections (perhaps with subclinical symptoms of disease) may be necessary to stimulate the production of heart-reactive antibodies. Only when there is a fully sensitized state to the cross-reactive antigens does the full disease complex appear (Zabriskie *et al.* 1970).

With respect to valvulitis, the sera of a number of patients with rheumatic fever and rheumatic heart disease have been shown to contain antibodies that bind to *N*-acetylglucosamine, the carbohydrate specific for the group A streptococcus. Patients with valvular disease have higher titres of this antibody than patients without valvular disease, and these antibodies persist for years after the initial attack (Dudding & Ayoub 1968). In contrast, the titres of patients without valvular disease decline rapidly after the initial rheumatic fever insult. The persistence of high titres in patients with rheumatic valvular disease may be related to the slow and sustained release of valvular cross-reactive glycoproteins, thus perpetuating the valvular damage.

Turning to the question of whether or not delayed hypersensitivity to haemolytic streptococci and their products may play a role in the pathogenesis of the non-suppurative sequelae, several investigators have agreed that hypersensitivity to streptococci and their products are a common occurrence in man, and increase in intensity with the age of the individual tested (Francis *et al.* 1967). These reactions were also more intense in rheumatic subjects than in non-rheumatic controls; the greatest number of positive reactions were obtained with autogenous streptococci, suggesting type-specificity to the reaction.

During the past 6 years our laboratory has been re-exploring this question of cellular reactivity to streptococcal antigens in patients with the known sequelae of streptococcal infection, namely rheumatic fever and post-streptococcal glomerulonephritis. In Trinidad these diseases occur simultaneously in the same age group, and this has offered us a unique opportunity to study both diseases in the same population. By using two *in vitro* assays for cellular reactivity to a given antigen, it was demonstrated that patients with acute rheumatic fever had an increased cellular response to certain streptococcal antigens when compared with acute nephritics. These antigens are primarly found in the membranes of those streptococcal strains commonly associated with rheumatic fever in Trinidad. This reaction persists for at least 2 years

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after the initial attack. Moreoever, there was no increased reactivity to those streptococcal antigens isolated from strains commonly associated with glomerulonephritis.

Although these results strongly suggest that there is a heightened response to streptococcal antigens in rheumatic individuals, the exact role played in the disease process by these sensitized cells remains unknown. The present finding that there is an abnormal cell-mediated response to membrane antigens, coupled with previous reports of an abnormal humoral response to the streptococcal membrane, argues strongly for a crucial role of this cell structure in the pathogenesis of rheumatic fever. The cross-reactive properties of these antigens might result in autosensitization to tissue antigens with cytotoxic effects in host tissues. This concept is in agreement with the histological findings of a large number of lymphocytic cells in and near the pathological heart lesions of rheumatic fever, and that these cells are in part composed of T_4 lymphocytic helper cells (Raizada *et al.* 1983).

Logistically it has been difficult to determine whether cytotoxic cells directed against human or other mammalian heart cells are operative in the disease process. In fact only two reports have appeared which directly addresses this question. The first is concerned with the work of Yang *et al.* (1977) in guinea-pigs, in which they demonstrated that animals sensitized to group A streptococcal membrane antigens produced aggressor lymphocytes that are more cytotoxic for embryo guinea-pig myofibre monolayers. The specificity of the reaction was attested to by the observation that neither group C nor group A cell-wall preparations elicited this response. Of particular interest to this discussion was their finding that addition of heart reactive antibody from the immunized guinea-pigs did *not* enhance the cytotoxic effect. The second piece of evidence is a small study conducted by Hutto & Ayoub (1977) in which a few patients with acute rheumatic fever carditis had circulating lymphocytes that were cytotoxic for cardiac myocytes derived from human atrial appendages, whereas controls were non-reactive in the assay system.

The second major antibody to be discussed concerns the immunoglobulin that binds to the caudate nuclear of brain and has been found to be present in the sera of patients with rheumatic chorea. The cross-reactive streptococcal antigen was originally delineated by Kingston & Glynn (1971) but only recently has its relevance to rheumatic fever chorea been elucidated. The work of Husby *et al.* (1976) has clearly shown that this antibody is present in at least half of all rheumatic chorea patients and was found in the cerebrospinal fluid of all five patients with acute rheumatic chorea (unpublished observations with H. Villarreal, Jr). Once again the rise and fall of this antibody in the sera of patients with acute chorea generally paralleled the disease process.

The 'streptococcal connection' of this antibody was firmly established by the observation that streptococcal membrane antigens (not cell walls) abolished the staining pattern of the caudate neuronal cells. The specificity of the reaction was also attested to by the fact that a *Lupus* anti-DNA antibody staining pattern was not diminished after absorption with streptococcal membrane antigens.

If, as was suggested at the outset, these microbial–host cross-reactions are occurring in many individuals, why do only a few individuals with streptococcal infections go on to develop rheumatic fever? In fact, starting with Dr Cheadle (1889) in his Harvean lectures, it was everyone's suspicion that individuals susceptible to rheumatic fever were in some manner genetically predisposed to contract the disease. However, numerous attempts to identify a genetic marker associated with the disease were unsuccessful or controversial. More recently,

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with the discovery of the close association of the major histocompatability complex and immune responses genes, a further unsuccessful search was made to link the frequency of an HLA antigen to the disease complex (Falk *et al.* 1973).

It was not until 1979 that workers at Rockefeller University in collaboration with M. E. Patarroyo in Colombia (Patarroyo *et al.* 1979) showed that a B cell alloantigen appeared to be closely associated with an increased susceptibility to rheumatic fever. Of potential importance to public health was the observation that the antigen was present in approximately the same number of rheumatic fever sufferers whether they were identified in New York or Bogota, suggesting a worldwide distribution of the antigen. Since then another antibody has been identified that recognized B cell antigens of most patients not identified with the original B cell marker. Thus the two antigens identify a subset of the normal population (15–20%) who are at high risk of contracting rheumatic fever. At present we still do not have a clear picture of what the presence of the marker means in relation to the disease process, and questions such as whether the antigen is present on other cells or tissues or whether the marker relates to the immune response to streptococcal antigens remains unanswered.

Other examples

As one might suspect, there are many other examples of cross-reactions between host tissues and microbial organisms. Foremost among these studies has been the elegant work of Perlmann *et al.* (1965). Over a period of years they have shown that *Escherichia coli* contains antigens that cross-react with antigens present in the human intestinal tract. The sera of patients with ulcerative colitis contain antibodies that react with both intestinal colon antigens and antigens from *E. coli*. An extremely important finding by those authors, and one that may have general applications in the field of microbial mimicry, is that the cross-reactions were observed primarily with foetal intestinal antigens (Lagercrantz *et al.* 1968). The fact that those observed cross-reactions have been seen in other inflammatory bowel conditions does not necessarily detract from these observations. As pointed out, many cross-reactions between microbes and host exist and certainly not all are deleterious to the host. It is only when these cross-reactions occur in a susceptible individual (as suggested for rheumatic fever) does the combination of microbial–host interactions in the genetically programmed individual give rise to a definite disease pattern.

The second example of another microbial–host interaction that is, to say the least, intriguing involves the reaction between *Klebsiella* spp. (and other similar organisms) and the HLA antigens B27, as described by Ebringer (1981) and Geczy *et al.* (1980). Ebringer's hypothesis is that antigens of certain strains of *Klebsiella* cross-react with antigens present in individuals bearing the HLA-B27 marker on their cells and that this cross-reaction in some manner leads to the disease ankylosing spondylitis. He favours the concept that it is shared antigenity between HLA antigens and microbial antigens that permits the persistence of the microbe, leading to an inflammatory reaction and disease. On the other hand, the evidence by Geczy *et al.* suggests that in some manner the microbial antigen binds preferentially to cells bearing the B27 marker of spondylitis patients and that this 'self+x' is the ultimate reason for the pathological damage. The importance of disproving or proving these hypotheses mentioned should not be underestimated because it would provide important clues about pathogenesis in one of the best examples of a close association of HLA antigens with a disease process.

A final example of microbial–host interactions in which the bacterial antigen apparently acts

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as a carrier of host tissue antigens is provided by the work of Feizi (1980). In this case it is felt that the mycoplasmal antigen absorbs the I antigen to its surface and may act as an adjuvant for presentation of the host antigens to the immune system. Irrespective of the exact mechanism involved, patients with mycoplasmal pneumonia often develop cold agglutinins, which may then cause immunopathological damage such as haemolytic anemia. While this is a rare occurrence in these patients, the phenomenon of a microbe presenting a host antigen to the immune system is important and may be operative in other bacterial–host symptoms.

Suppression of the immune response

The second mechanism whereby pathogenic organisms achieve pathological damage is in somehow circumventing or suppressing the host's immune response. During studies of acute post-streptococcal nephritis in Trinidad, we noted that the blastogenic response to certain streptococcal antigens in nephritis patients was depressed compared with controls. In view of Baldwin's observations (1974) that patients who contracted acute post-streptococcal glomerulonephritis (APSGN) after the age of 10 years had a much worse prognosis, the observed response to the streptococcal antigens in the Trinidad patients was broken down into those who had nephritis before age 10 and those who had APSGN after 10 years of age. The acute nephritic patients older than 10 years were quite similar to controls in their response to the streptococcal antigens. In marked contrast, patients over the age of 10 years had definite cellular suppression to these antigens.

Suspecting that the cellular suppression to these antigens might be related to an adherent cell population we designed experiments to test the reaction to these antigens after the removal of the adherent cell population. The removal of the adherent cell population markedly enhanced the observed response of nephritic patients to these streptococcal antigens. Reintroduction of these adherent cells into the assay system promptly depressed the response to the streptococcal antigens. Because we had noted that nephritics have elevated levels of T gamma (Tg) cells during the acute stages of the disease, attempts were made to remove Tg cells from the mononuclear cell populations. The results of these experiments were inconclusive because the response to streptococcal antigens was abrogated in both nephritics and controls after the removal of Tg cells. These results suggest that the removal of Tg cells resulted in the simultaneous non-specific removal of antigen-reactive cells in the text system.

Other examples

Work by a number of investigators has demonstrated that cellular suppression to various specific and non-specific mitogens occurs in a number of disease states involving bacteria, viruses and protozoa. In the context of this discussion, I shall, however, limit my remarks to examples involving indirect cellular suppression by bacteria in disease states. In this respect, among those best studied has been the observed cellular suppression in tuberculosis. Katz *et al.* (1979) noted the appearance of suppressor monocytes in untreated tuberculosis patients, associated with a cellular suppression to both pokeweed mitogens and mycobacterial antigens. Removal of this adherent non-T cell population resulted in a marked increase in the cellular response to these antigens. Treatment of these patients with anti-tuberculosis medications resulted in a complete return of normal cellular response in about 4–6 weeks.

More recently, investigators of leprosy lesions (Wesley *et al.* 1982) have found that a large

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proportion of T cells in the cutaneous lesions of lepromatous patients were predominantly of the T_8 (suppressor cell) series and essentially devoid of T_4 (helper) cells. In contrast, turberculoid leprosy had predominantly T_4 cells and the authors suggested that these cells may have a strong influence on the microbicidal activity of the macrophages in the lesions. These studies also suggest that factors secreted by those macrophages laden with the organisms may lead to a specific signal for one or another T cell population. If true, these observations have important implications because they indicate that it is the microbe itself that directs an immune cell to release lymphokines, which direct the introduction of a specific suppressor or helper cell into the actual lesion.

Direct immunopathological damage

The final example provided by the group A streptococcus is concerned with the release by the oganism of certain substances that appear to have rather specific biological properties leading to immunopathological damage in the host. In terms of post-streptococcal nephritis it appears that the particular substance secreted by the group A streptococci is a protein that is primarily elaborated by those strains associated with nephritis. The elegant work of Villarreal *et al.* (1979) has demonstrated that it has a molecular mass of 46 kDa and is present in most of the biopsy specimens obtained from patients with APSGN.

Although the presence of the protein in the biopsy specimens argued strongly for a causal association of the protein with the disease process, the question of how the protein becomes located in the kidney has been the subject of our recent experiments. Because our previous studies (van de Rijn *et al.* 1978) had demonstrated that patients with APSGN had high levels of circulating immune complexes, the question of whether or not these complexes contained streptococcal antigens as an integral part of the circulatory complex was investigated. The approach was to first isolate the complexes by using polyethylene glycol precipitation of the complex followed by column isolation of the purified complex. These complexes were then injected into rabbits in order to produce anti-immune complex antibodies. These studies indicated that the anti-immune complex rabbit serum contained antibodies that reacted with streptococcal extracellular preparations obtained from those strains associated with nephritis. In contrast, rabbit antisera made against circulating complexes isolated from acute rheumatic fever patients reacted with antigens secreted by rheumatogenic strains and the patterns were quite different in both groups.

More importantly, the use of 'rocket' electrophoresis techniques on isolated nephritic complexes and rabbit serum raised against streptococcal extracellular products demonstrated that streptococcal antigens are definitely within the circulating complex and that the removal of the complex by means of protein A anti human globulin sera resulted in both the removal of the complex and removal of the streptococcal antigens bound to the complex. A comparison of the major antigen within the complex with the 46 kDa antigen described by Villarreal *et al.* indicate they are the same antigens.

Thus it appears that extracellular antigens unique to those strains associated with the disease are present both in the circulating complex and are deposited in the glomerular tissues of these patients. It is my belief that this antigen has unique biological properties, some of which include its ability to trigger the alternative pathway of complement, and it is this property alone or in combination with the circulating complex that causes the pathological damage. This could

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explain the well known observation that streptococcal antigens and complement appear early in this post-streptococcal glomerular lesions at a time long before host antibody appears in the lesion.

Other examples

The list of exogenous bacterial antigens that have been implicated in various forms of nephritis is long and has been recently reviewed by Villarreal *et al.* (1982). In many of the other bacterial antigens a pathological picture of proliferative nephritis similar to that seen in post-streptococcal glomerulonephritis is also seen. Because many of these bacterial antigens may trigger the alternative pathway of the complement, the mechanism for inducing pathological damage to the kidney may be similar in all post-bacterial glomerulonephritis.

Lest the reader be left with the impression that bacterial antigens act only by complement activation or complex formation, it has also been proposed that certain organisms secrete sialidases that could alter the composition of host immunoglobulins, rendering them 'altered' and thus perceived as foreign. Complexes of altered immunoglobulin anti-immunoglobulin antibodies would then be formed and it is these complexes that eventually lend to the pathological damage. Evidence supporting this concept is found in the observation that cryoglobulins are present in a large proportion of APSGN patients and that certain strains of group A streptococci produce sialidases capable of removing sialic acid from IgG molecules (McIntosh *et al.* 1975).

Conclusions

It is clear from the above that microbes employ a number of different immunological pathways leading to pathological damage to the host. Although I have drawn from examples of pathways used by the group A streptococcus, it would appear that other microbes use similar mechanisms to initiate or perpetuate (or both) their immunopathological damage. Thus for cross-reacting antigens many microbes have antigens that cross-react with relevant tissue antigens. One is led to believe that in most cases these cross-reactions are harmless. In the susceptible individual, however, these cross-reactions either at a cellular or humoral level, or both, leads to immunopathological damage. Furthermore, the genetic make-up of the individual has a strong influence on the outcome of these bacterial–host interactions either by virtue of antigen recognition (or lack of it), regulation of the immune response or as receptors for antigens.

With respect to immunosuppression of the host's defences by bacteria, both non-specific and specific immunosuppressive mechanisms are operative. However, it now appears that either cellular or extracellular products of bacteria can redirect cells involved in the host's immune system to cause immunosuppression of the host's defences. These tactics are often subtle and work on one or more of the cells implicated in the immune process, leading to the release of factors that then suppress or activate the subsets of the lymphoid series. Whether or not this activation or suppression also maintains the chronic state of the disease process remains unanswered as yet but, if true, has broad immunopathological implications.

Finally, organisms may secrete certain substances that either directly or indirectly cause immunopathological damage by the deposition of circulating complexes in target organs. In this context the nature of the bacterial antigen within a complex appears to be of paramount importance. Thus in post-streptococcal nephritis, a nephritis-associated protein with some as

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yet undefined affinity for a kidney component appears to be an important factor in initiating the disease. In contrast, rheumatic fever complexes similar in size, duration and level appear to cause damage in different target organs. Furthermore, this type of antigen-specific complex might explain why some complexes are injurious and some are not. Thus the levels of circulating complexes may not in themselves cause any pathological damage in some cases, whereas others may have specific target sites. To emphasize the subtleties of these pathways, the possibility that certain bacteria excrete substances that indirectly cause damage by virtue of specific enzymes directed towards a given host component should not be minimized.

In conclusion, these microbial–host pathways are often complex and in many instances do not have suitable experimental models for intensive study. Yet the challenges of more fully understanding the immunopathological consequences of a given bacterial–host interaction as it occurs in man make the study of these disease processes worth our continued efforts.

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Discussion

Soad Tabaqchali (*Department of Microbiology, St Bartholomew's Hospital, London, U.K.*). I should like to comment on the role of intestinal bacteria and the pathogenesis of inflammatory bowel disease. The work of Perlmann *et al.* (1965, 1967), and Lagercrantz *et al.* (1968) suggested that *E. coli* O14 had a common enterobacterial antigen that reacted with human colonic mucosa and showed that patients with ulcerative colitis (UC) and Crohns disease (CD) had an increased incidence of antibodies to O94. Also Shorter *et al.* (1970) and Bull & Ignaczak (1973) demonstrated that antigens derived from *E. coli* O14 and O119 K69 (B14) induced lymphocytes to become cytotoxic to colonic mucosa. These findings led to the suggestion that these specific organisms were causally involved in the pathogenesis of inflammatory bowel disease (IBD).

To test this hypothesis and because of the variety of *E. coli* serotypes present in the human intestinal flora, we screened sera from 30 patients with IBD (16 with CD and 14 with UC), for the presence of antibodies against O-antigens derived from 159 *E. coli* serotypes and compared these with sera from 16 matched control subjects.

Most patients with IBD had agglutinating antibodies to a higher number of *E. coli* O antigens and in higher titres than the control group. The mean number of positive agglutinins was 13.8 for CD, 7.9 for UC and 1.5 in controls. Eight patients with IBD and arthropathy had antibodies to fewer O-antigens (mean 3.2). The antibodies were in the IgG and IgM, in titres corresponding to the original values. No specific serotypes were associated with IBD. The commonest serotypes were those shown to be associated with invasive properties. Antibodies to O14 were detected in five patients and to O119 in none.

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Immunofluorescence studies with intestinal biopsies from these patients and specific antisera against the positive *E. coli* O-antigens, detected the presence of the antigens predominantly in the goblet cells of the colonic mucosa.

On the basis of these findings, we suggested that the presence of the high numbers of *E. coli* antibodies in patients with IBD is secondary to the disease process and is unlikely to be causally involved in the pathogenesis of the disease, but the high levels of antigens and antibodies in the serum may play a role in the perpetuation of the disease and in the pathogeneis of extra-intestinal complications (Tabaqchali *et al.* 1978).

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J. B. Zabriskie. I think these findings are quite interesting and tend to reinforce my original theme that there are many inflammatory bowel conditions in which antibodies to *E. coli* develop and that antigens may well be still present in various target organs. Also one may even see these *E. coli* antibodies exhibit cross-reactions to tissues in normal or diseased individuals. What has to be shown is that these antibodies do cross-react with the target tissue and are absorbed by the specific microbial and target tissue, and if possible one should demonstrate either cellular or humoral (or both) reactions to the target organ (cytotoxic antibody or cells). The key is not the cross-reaction but what makes the cross-reaction damaging. I do not think that these findings necessarily negate Perlmann's original hypothesis or findings.

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