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Immunological Aspects of Leprosy with Special Reference to Autoimmune Diseases

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Leprosy, particularly lepromatous leprosy, is associated with a multitude of (auto) immune aberrations, and its clinical features also have much in common with the collagen diseases. Immunopathological studies of the 2 groups of diseases may thus elucidate the basic mechanisms of both.

The reported evidence for a genetically determined hyporeactivity of cell-mediated (CM) immunity in lepromatous subjects is reviewed; most, but not all, of the findings fit such a hypothesis well. The possibility remains that the observed hyporeactivities may be secondary to direct effects of Mycobacterium leprae. *Evidence for a general hyperreactivity of the antibody-mediated (AM) immunity in lepromatous leprosy is then reviewed and considered to be fragmentary.*

The concept and general criteria of autoimmunity are discussed briefly and the high incidence in lepromatous leprosy of various (auto)immune aberrations, resembling those in systemic lupus erythematosus (SLE) and in rheumatoid arthritis is reviewed. Although autoantibodies are not likely to be directly deleterious to the host, immune complexes containing autoantibodies may be pathogenic.

Mixed cryoimmunoglobulins, consisting of 2 (IgG–IgM or IgG–IgA) or 3 immunoglobulins, and occasionally also containing measurable amounts of complement components, have recently been encountered in SLE and its variants and also in a number of microbial diseases with autoimmune features (syphilis, streptococcal nephritis and endocarditis, mononucleosis, Mycoplasma pneumoniae *pneumonia). They may represent circulating immune complexes, analogous to the IgM (IgA) rheumatoid factors in combination with their IgG reactants. In leprosy also, the existence of pathogenic immune complexes is indirectly suggested by mixed cryoglobulinemia and further by a number of other features reviewed in this article.*

Leprosy is an infectious disease associated with a multitude of immunological aberrations. Both the cell-mediated (CM, " thymus-dependent ") and the antibody-mediated (AM, " bursa-dependent ") immune systems are involved (Oort & Turk, 1965; Parrott, de Sousa & East, 1966; Cooper, Gabriel & Good, 1967). The responses fall predominantly within the types III and IV of the widely used classification of allergic responses by Gell & Coombs (1963) (see Annex). Many of the AM responses are similar to those regarded as typical of the so-called collagen diseases, say, systemic lupus erythematosus (SLE) and rheumatoid arthritis.

The clinical features of leprosy also have much in common with collagen diseases. In fact, as has been pointed out by Matthews & Trautman (1965), were leprosy not present in patients with, for example, polyarthritis, subcutaneous nodules, lymphadenopathy, hepatosplenomegaly, butterfly facial lesions, etc., a collagen disease would immediately be suspected on the basis of clinical findings.

The immunological aberrations in leprosy, with the exception of the lepromin reactivity, have only recently been subjected to more extensive analyses by modern immunological techniques. Similar aberrations in collagen diseases, on the other hand, have for some decades already been studied intensively, and much valuable information has been collected on the underlying mechanisms and their pathogenic role.

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The etiology of the collagen diseases, however, remains unknown, whereas that of leprosy, with reasonable likelihood, is infection by *Mycobacterium leprae*. Thus, the information extracted from immunological studies of leprosy, on one hand, and of the collagen diseases on the other, might possibly be mutually useful in elucidating the basic mechanisms of both.

In the following report the main emphasis is laid upon descriptions of the AM immune responses and reference is made to analogies between the AM responses in leprosy and in collagen disease.

IMMUNE MECHANISMS

Cell-mediated immunity

The lepromin reactivity of the Mitsuda type is generally regarded as an example of delayed cell-mediated hypersensitivity to the bacillary component of lepromin. To the arguments favouring this view, as given recently in a comprehensive review by Hart & Rees (1967), may now be added a claim of successful passive transfer of lepromin sensitivity by lymphoid cells from sensitized guinea-pigs to isologous recipients (Goihman-Yahr, Raffel & Ferraresi, 1968).

In addition to lepromin negativity, other phenomena compatible with a depression of CM immunity have been observed in lepromatous leprosy. These include the following:

(1) Impaired reactivity to unrelated skin test antigens, such as *Candida albicans*, 2, 4-dinitrochlorobenzene and picryl chloride (Buck & Hasenclever, 1963; Guinto, 1968; Bullock, 1968; Waldorf et al., 1966).

(2) Impaired lymphocyte transformation response to phytohaemagglutinin, to *Myco. leprae* and *Myco. tuberculosis* antigens (Dierks & Shepard, 1968), and to streptolysin O (Sheagren et al., 1967), in cultures from peripheral blood.

(3) Delay in the rejection of skin homografts up to a maximum of 70 days in 4 patients with lepromatous leprosy grafted with skin from donors with a similar type of disease (Job & Karat, personal communication, cited by Hart & Rees, 1967).

(4) A higher than normal incidence of local necrotic and disseminated reactions following smallpox vaccination (Turk, personal communication).

(5) A histological picture of lymph nodes from 9 patients with lepromatous leprosy, corresponding closely to that seen in guinea-pigs treated with anti-lymphocyte serum (Turk & Waters, 1968), i.e., a replacement of the lymphocytes in the paracortical (thymus-dependent) area by reticulohistiocytes (Turk & Willoughby, 1967). The germinal centres and the medullary cords (bursa-dependent area), however, were normal.

These findings all fit well the hypothesis of genetically determined general depression of the CM immunity (Turk, 1968 and personal communication; Newell, 1966).

There are, however, other features in lepromatous leprosy not so compatible with such a hypothesis. First, the distribution of the incidence of tuberculin positivity (Mantoux) in different age-groups of patients with lepromatous leprosy is the same, or only slightly lower, than that found in the general population (Hart & Rees, 1967; Guinto & Mabalay, 1962) [1] in spite of the common antigenic determinants shared by *Myco. leprae*, *Myco. tuberculosis* and a variety of other mycobacteria and *Nocardia* (Rees et al., 1965; Norlin et al., 1966; Estrada-Parra, Rojas-Espinosa & Reyes-Gomez, 1968; Navalkar, 1968).

Secondly, in lepromatous leprosy, BCG-induced weak lepromin reactivity is 11%–50% in different studies, while converting normally to tuberculin reactivity (Hart & Rees, 1967).

Thirdly, CM immunity is generally believed to play a central part in the resistance not only to mycobacterial infections (Lurie, 1964; Salvin, 1963), but also to fungal and viral infections and in resistance to tumours. Yet, tuberculosis is no more common among patients with lepromatous leprosy than in the general population (Khoshoo, 1968), nor is malignancy (Oleinick, 1968) with the possible exception of tumours of the skin (Michalany, 1966). The same seems to apply to the incidence of viral infections.

Thus, the evidence so far produced for the attractive thesis of a genetically determined general depression of the CM immunity in lepromatous leprosy cannot yet be regarded as conclusive. The possibility remains that this depression may be secondary to direct effects of *Myco. leprae* infection (Guinto, 1968), resulting, for instance, in specific tolerance of the CM system while the AM system remains

[1] See also Bechelli, L. M. (1965) *A review of present literature on the immuno-allergic correlation between tuberculosis and leprosy*; unpublished WHO working document PA/66.59. A limited number of copies of this document is available to persons officially or professionally interested on request to Leprosy, Division of Communicable Diseases, World Health Organization, 1211 Geneva, Switzerland.

specifically active. Burnet (1968) has recently suggested that such a state of split tolerance may be caused in subacute sclerozing panencephalitis by infection of the thymus with the measles virus.

Further research should include extension of the most challenging histological studies of 9 lymph nodes taken from patients with bacilliferous lepromatous leprosy (Turk & Waters, 1968) to bacillus-free nodes from both polar ends of the spectrum, and particularly to nodes from lepromin-negative subjects without clinical leprosy and to the thymus gland. In order to ascertain the inclusion of subjects genetically lacking the " N-factor " (Newell, 1966), a sufficiently large number of samples should be collected from adults living in heavily infected endemic areas.

Antibody-mediated immunity

In sharp contrast to the hyporeactive state of the CM immune responses in lepromatous leprosy, data compatible with a general hyperreactivity of the AM immune responses in lepromatous leprosy have been published.

In addition to precipitating antibodies to a variety of mycobacterial antigens (Bullock, 1968; Rees et al., 1965; Estrada-Parra, Rojas-Espinosa & Reyes-Gomez, 1968; Navalkar, 1968) found in the sera from lepromatous patients and in much lesser degree in those from patients with tuberculoid leprosy, typhoid vaccination induced higher agglutinin titres in lepromatous patients than in normal controls (Almeida, Brandao & de Lima, 1964). The titre level of isoagglutinins (Buck & Hasenclever, 1963), of agglutinins against *Candida albicans* (Buck & Hasenclever, 1963), and the antistreptolysin-O titres (Eliasberg, 1911) were also higher in non-vaccinated patients with lepromatous leprosy than in controls.

Depressed level of haemolytic complement (Eliasberg, 1911; de Azevedo & Melo, 1966) and its components (Bonomo et al., 1967) have also been reported to occur particularly in the reactional phases of leprosy, and interpreted as indirect evidence for complement-fixing antigen–antibody interactions *in vivo*.

The evidence for a general hyperreactivity of the AM immune mechanisms, however, is rather fragmentary, and only too susceptible to criticism. As to the effects of typhoid vaccination (Almeida, Brandao & de Lima, 1964), the lepromatous leprosy series and the control series were not comparable as regards age. The probable presence of the rheumatoid factor in many of the lepromatous leprosy sera (Bonomo & Dammacco, 1968), furthermore, may have exerted an enhancing effect upon the agglutinin titres, as it is known to do in rheumatoid sera (Wager, 1950).

The specificity of the elevated antistreptolysin-O titres, again, was not ascertained by removal of the lipids known to cause non-specific increase of the antistreptolysin titres in dysproteinaemic states, such as hepatitis and liver cirrhosis.

And yet, taken together, these pieces of evidence constitute a mosaic, suggesting a possible general hyperreactivity of the AM immune responses. The presence of large amounts of mycobacterial adjuvant in the tissues of lepromatous patients, in fact, would suffice to explain such a hyperreactivity without any additional hypothesis of a primary constitutional host-dependent hyperreactive make-up of the lepromatous host.

More extensive and controlled vaccination trials with a variety of immunogens unrelated to *Myco. leprae* may throw more light upon these considerations.

In this context, it may be mentioned that a similar dissociation of the CM and the AM immune responses has been observed in rheumatoid arthritis (Houba et al., 1964).

Autoimmune serum factors

The concept of autoimmunity has become increasingly difficult to define. The original term " autoimmune disease" implies direct attack by immunological factors on the tissues towards which the immunological factors are directed. However, in many conditions, traditionally accepted as respectable members of the family of autoimmune states, say, SLE nephritis, the tissue damage actually seems to be caused by antigen–antibody interactions unrelated to the target tissue (see below). The role of the target tissue, so to speak, seems to be limited to that of an innocent bystander caught up in an immunological event not of its own making (Vaughan, 1965).

However, the term " autoimmunity " being widely accepted, it does not seem wise to become hopelessly entangled in semantics, e.g., by suggesting replacement of this traditional term by others, such as auto-allergy, auto-aggression or autosensitivity. A better choice, according to the advice of Dixon (1967), is to concentrate upon the different basic, and possibly pathogenic, immune mechanisms operative within this fairly ill-defined group of diseases.

To this end, the classification of the allergic reactions into 4 types by Gell & Coombs (1963) is applicable, and for practical diagnostic and therapeutic purposes, the " markers of autoimmunity " as

proposed by Mackay & Burnet (1963) have proved most useful. The two classifications, slightly modified, are given in the Annex.

Leprosy, particularly its lepromatous type with erythema nodosum leprosum (ENL) reactions, meets most of the requirements for autoimmunity as laid down by these markers. Its infectious etiology, however, has excluded it from " full membership " in the family of autoimmune diseases *sensu stricto*.

Hypergammaglobulinaemia, for example, is a common finding in leprosy (Matthews & Trautman, 1965; Bonomo & Dammacco, 1968; Bonomo, Dammacco & Gillardi, 1968). A high, SLE-like incidence of various autoantibodies or " autoimmune serum factors " has been reported by several authors to be a feature of lepromatous leprosy. These observations have recently been discussed and summarized by Bonomo & Dammacco (1968), who also made a comparison between the autoimmune patterns in leprosy and SLE. The approximate incidences in lepromatous leprosy (and SLE) were for antinuclear factors 30% (95%), for LE-cells 8% (95%), for rheumatoid factors 50% (70%), for thyroglobulin antibodies 40% (20%), for cryoglobulins 95% (50%), for biologically false positive tests for syphilis 70% (20%), and for low complement levels 50% (50%). Thus, a broad serological overlap between SLE, the autoimmune disease *par excellence*, and lepromatous leprosy has been clearly established, and as to the incidence of the rheumatoid factor (RF), also between lepromatous leprosy and rheumatoid arthritis. An issue of greater importance than the mere presence of the various autoimmune factors in the circulation, however, is their possible role in the pathogenesis of the leprosy lesions.

Circulating autoantibodies in general do not seem to be directly deleterious to the host, with the notable exception of autoantibodies causing red cell destruction in autoimmune haemolytic anaemia. In leprosy, cold agglutinins against human O cells have not been found (Matthews & Trautman, 1965). On the contrary, a protective role has been ascribed to some autoantibodies, e.g., the RF (Vaughan, 1959; Houba et al., 1964). The best example of a " physiological ", non-aggressive autoantibody is the immunoconglutinin, which fulfils all the criteria for an autoantibody against autologous altered complement, and yet with reasonable likelihood enhances the resistance, at least of mice, to a variety of bacterial infections (Coombs, Coombs & Ingram, 1960).

By analogy with what has been said above, the circulating autoantibodies are not likely to play directly any major part in the pathogenesis of leprosy.

As to those immunological mediator mechanisms, possibly responsible for tissue lesions in leprosy, the experimental observations on the phlogogenic capacity of soluble antigen–antibody complexes and the rapidly accumulating evidence that similar mechanisms are operative in SLE nephritis are of greatest interest, and will be dealt with below.

Antigen–antibody complexes as a cause of tissue damage

Experiments in animals have clearly established that intermediate-sized antigen–antibody complexes formed in moderate antigen excess and therefore soluble are able to cause tissue lesions that neither antigen nor antibody alone causes, if administered separately (Dixon, 1962-63, 1965; Weigle, 1961). Pathogenetically, the simplest complex-induced lesion is the Arthus reaction, a localized, acute necrotizing vasculitis. The complexes, once deposited in vessel walls, *via* their reaction with complement, attract polymorphonuclear leucocytes which, in turn, release various enzymes injurious to vessel walls. In the sites of tissue lesions, the antigen in question, host immunoglobulin (probably the corresponding antibody) and complement components can be shown by immunofluorescent methods. More complex, but closer to the situation in systemic human disease, is the classical " one-shot " serum sickness.

Most applicable to systemic human disease, however, is the experimental antigen–antibody complex nephritis, first induced by Dixon and his co-workers (Unanue & Dixon, 1967) in rabbits by intravenously injecting daily for prolonged periods small amounts of heterologous serum protein (BSA, HSA, BGG, HGG). In those rabbits making antibody responses too small to cause elimination of antigen but sufficient to result in the formation of soluble antigen–antibody complexes, a chronic progressive glomerulonephritis developed. Again, antigen, host gammaglobulin, and host complement were found concentrated in the thickened glomerular basement membranes. By electron microscopy, a lumpy, dense deposit was seen along the outer aspect of the basement membrane.

During the last few years, evidence has been accumulating that analogous mechanisms are responsible for the nephritis (Koffler et al., 1967; Christian, Hanauer & Pincus, 1967) and the skin lesions (Kunkel & Tan, 1964) in human SLE as well as for the spontaneous lupus-like glomerulonephritis in

NZB and related strains of mice (Dixon, Edgington & Lambert, 1967).

In addition to demonstrating gammaglobulin and complement components by immunofluorescence at the sites of lesions, gammaglobulin-containing acid eluates from the SLE kidneys and NZB mouse kidneys contained high concentrations of antinuclear antibodies. Nuclear antigens deposited along the glomerular capillary walls also were detectable by immunofluorescence (Dixon, Edgington & Lambert, 1967; Koffler et al., 1967). Furthermore, nuclear DNA antigen was found in the serum of NZB mice by the Ouchterlony technique (Dixon, Edgington & Lambert, 1967), and in the serum of patients with SLE nephritis a replacement of antinuclear antibody by circulating nuclear antigen has been observed in association with aggravation of this form of renal disease (Tan, Schur & Kunkel, 1965).

These findings suggest that the possibly pathogenic, complement-fixing immune complexes in human SLE, and in the SLE-like nephritis in NZB mice, are composed of nuclear antigens and the corresponding antibodies.

Other indirect pieces of evidence for the presence of immune complexes (or other complement-fixing aggregates) in the circulation of patients with SLE are the depression of the C'_3 component (β_{1C}–β_{1A} globulin) and of the total haemolytic complement activity occurring during periods of clinical exacerbation, the presence in fresh plasma of altered complement component C'_{3A} (β_{1A}), and the anticomplementary activity of many SLE sera (Christian, Hanauer & Pincus, 1967).

Recently, Penttinen and his co-workers have reported most interesting observations on the capacity of minute amounts of certain soluble viral antigen–antibody complexes to aggregate platelets, and they have produced evidence suggesting a pathogenic role for rubella antigen–antibody complexes in the post-rubella thrombocytopenic purpura (Penttinen & Myllylä, 1968; Myllylä et al. 1969). From their results, it appears possible that virus antigen–antibody interactions may be important also in other manifestations and late complications of viral infections.

Attempts at direct identification and isolation of the immune complexes from human sera, however, have met with difficulties. Ultracentrifuge analyses, it is true, have yielded evidence for the presence of soluble immune complexes, e.g., in the sera from patients with SLE, purpura, rheumatoid arthritis, Sjögren's syndrome, and subacute bacterial endocarditis. This method, however, is elaborate, insensitive and, in addition, it does not make certain the antigen–antibody nature of the gammaglobulin complexes. The recent findings on cryoglobulinaemia, to be reviewed below, may have opened new possibilities to attack these problems.

Cryoimmunoglobulins

Cryoglobulins, by definition, are serum globulins precipitating in the cold (+4°C) and soluble at body temperatures (Lerner, Barnum & Watson, 1947). Cryoimmunoglobulins most likely to represent circulating immune complexes are those composed of 2 (IgG–IgM or IgG–IgA) or 3 (IgG–IgM–IgA) polyclonal immunoglobulins and occasionally also containing measurable amounts of complement components, primarily C'_{3A} (β_{1A}).

Mixed (IgG–IgM and IgG–IgA) cryoglobulinaemia has been recently encountered in association with SLE, with its variants, such as the purpura–arthralgia–Raynaud phenomenon–glomerulonephritis–rheumatoid factor syndrome, with rheumatoid arthritis, with polyarteritis nodosa and with other pathological conditions probably caused by underlying autoimmune (immune complex) mechanisms (Hanauer & Christian, 1967; Meltzer & Franklin, 1966; Peetoom & Loghem, 1965; Wager, Mustakallio & Räsänen, 1968; Wager et al., 1967; Wager & Räsänen, 1969).

The simultaneous occurrence of mixed cryoglobulins and a number of recognized autoantibodies (cold agglutinins, rheumatoid factors, antinuclear antibodies) in syphilitic infections (Mustakallio, Lassus & Wager, 1967; Wager et al., 1967), post-streptococcal nephritis (van Loghem, 1966), staphylococcal osteitis, subacute bacterial endocarditis and *Mycoplasma pneumoniae* pneumonia (Wager, unpublished data), infectious mononucleosis and cytomegalovirus–mononucleosis (Wager et al., 1968a), and in other diseases related to microbial infection, is interesting, considering a possible causal relationship between infection on the one hand and autoimmunity on the other.

Recently, Wager and his co-workers have isolated and characterized 120 cryoglobulins composed of IgG and IgM or IgA or both, out of which 19 also contained β_{1A} (Wager & Räsänen, 1969). In addition to cryoimmunoglobulinaemia, other immunological aberrations were encountered in the sera of these 120 patients. Cold agglutinins were found in 58% (normal incidence 21%), rheumatoid factor activity in 30% (4.1%), and antinuclear antibodies in 35%

(0.5%) of the cryoglobulinaemic sera. The various serological activities were recovered in several instances from the isolated cryoglobulins, and were in some instances found in these only. Most of the isolated cryoglobulins, their parent sera and the serum supernatants left after removal of the cryoprecipitate, possessed a strong anticomplementary activity, similar to that shown under the same experimental conditions by known specific precipitates of human and rabbit origin (Wager et al., 1967, 1968a, 1968b; Wager, Mustakallio & Räsänen, 1968). Heating at +56°C in most instances abolished or greatly reduced the anticomplementary effect of the cryoglobulins (and their parent sera), and probably had some similar action upon the diphtheria toxoid–human IgG–IgM–IgA antitoxin precipitate, but it had no measurable effect upon the egg albumin–rabbit IgG antibody precipitate formed by Ea and hyperimmune rabbit antiserum. Three human polyclonal IgG preparations were not anticomplementary, but became slightly anticomplementary following treatment at +56°C and highly so when treated at +63°C. Human IgA and IgM preparations were not anticomplementary and were not affected by treatments at +56°C or +63°C.

The IgG–IgM cryoglobulins have been subjected to some dissociation and reprecipitation analyses employing acid buffers and gel-filtration on Sephadex G-200 (LoSpalluto et al., 1962; Peetoom & van Loghem, 1965; Wager et al., 1967). Neither of the dissociated components possessed cryoprecipitating capacity alone, but reprecipitation in the cold occurred when the components were brought together. The IgM component behaved as the "antibody" by accepting as its cryoprecipitating "antigen" any human (and even rabbit) IgG, whereas the IgG component for cryoprecipitation required the IgM component of the cryoglobulin, and failed to cryoprecipitate with IgM from other sources. The evidence, admittedly mainly circumstantial, for the immune complex nature of the mixed cryoimmunoglobulins may be summarized as follows:

(1) The dissociation and reprecipitation patterns of the IgG–IgM cryoglobulins resembling the behaviour of the IgM rheumatoid factor ("anti-IgG factor") reacting with its human IgG "antigen" and cross-reacting with heterologous IgG (Aho, 1961). This analogy is further supported by the presence in practically all of the mixed cryoimmunoglobulins hitherto reported of IgG as one of the components, the other components being either IgM or IgA or both.

(2) The thermolabile anticomplementary effect of the isolated cryoglobulins.

(3) The presence of complement components in the isolated cryoglobulins.

(4) The various (auto)antibody-like activities of the isolated cryoglobulins.

(5) The presence of DNA in some of the cryoglobulins isolated from sera of patients with SLE (Forsén & Barnett, 1968).

It seems possible that only a part of the circulating immunocomplexes possess a cryoprecipitating property as the various serological activities, including the anticomplementary activity of the whole sera, mostly diminished only slightly or non-measurably following removal of the cryoprecipitate.

Assuming that the mixed cryoimmunoglobulins represent complexes composed of "IgG-antigen" and "IgM and/or IgA-antibody" components, 2 questions immediately arise. First, what is the primary event making the autologous IgG autoantigenic? Second, are these immune complexes, all or a part of which possess cryoprecipitating properties, pathogenic like those believed to be responsible for the lesions in the experimental nephritis and in SLE?

As to the first question, the primary events might well be similar to those probably responsible for the elaboration of the rheumatoid factor, i.e., a sustained stimulation with some antigen or antigens, leading first to specific IgG antibody formation against the primary antigen or antigens and then, via antigen–IgG antibody complex formation to unfolding of the IgG molecule and exposure of hidden autoantigenic determinants on the latter. These exposed IgG-determinants would then secondarily induce the elaboration of anti-IgG autoantibodies, usually of the IgM variety (Christian, 1965).

The primary inciting antigen then would likely be the corresponding causative agent (treponeme, streptococcus, cytomegalovirus) in infectious diseases, unknown in rheumatoid arthritis (perhaps mycoplasma or virus), and *Myco. leprae* antigens in leprosy. There is clinical as well as experimental evidence suggesting that mechanisms as delineated above may be responsible for the production of rheumatoid factors (Abruzzo & Christian, 1961; Aho et al., 1962; Aho & Wager, 1961) and also of mixed cryoimmunoglobulins (Catsoulis, Franklin & Rothschild, 1965; Wager & Räsänen, 1969).

As to the second question, not much is actually known about the pathogenicity of the cryoimmuno-

globulins proper. The most suggestive evidence is indirect and comes from the clinical observations of the various manifestations of cold sensitivity associated with the purpura–arthralgia–Raynaud–rheumatoid factor syndrome. In skin biopsy specimens taken from a few patients with this syndrome, immunoglobulins (IgG and IgM) and complement (β_{1A}) have been found in the vessel walls by immunofluorescent methods, and histologically Arthus-like changes were seen (Miescher, Paronetto & Koffler, 1966). Nothing is known, however, of the possible cryoprecipitating properties of these deposited immune complexes.

In most other conditions associated with cryoimmunoglobulinaemia, the patients do not exhibit any overt clinical signs of cold sensitivity (Wager et al., 1968a, 1968b). Intradermal injection of autologous IgG–IgM cryoglobulin isolated from a patient with *M. pneumoniae* pneumonia and skin rash did not cause any immediate or delayed local reaction (Wager, unpublished data).

There appears to be a considerable variation in the solubility patterns of individual cryoglobulins. Most of them were readily redissolved *in vitro* at +37°C, but some, primarily those isolated from patients with the purpura–cold sensitivity syndrome, were only partially soluble at +37°C (Wager, unpublished data). The same applies to the temperature and pH requirements for dissociation of the components, as shown by preparative procedures (Wager, unpublished data) and also by analytical ultracentrifugation of some cryoglobulins (LoSpalluto et al., 1962; Wasastjerna et al., 1967). Thus, some cryoglobulins seem to be composed of more " avid " components than do others. The size of the immune complexes, shown under experimental conditions to be of paramount importance for their localization in vascular structures (Cochrane, Hawkins & Kniker, 1967), may be added to the list of factors probably determining the *in vivo* behaviour of the cryoimmunoglobulins also.

Finally there remains the possibility that the IgM or IgA components or both of the circulating cryoglobulins, by analogy with what has been said about the rheumatoid factors above (see above, pp. 795-796), are exponents of defence mechanisms of the host against exogenous or endogenous antigens in combinations with their corresponding IgG antibody. If so, then the mixed cryoglobulins might represent intermediate products of host defence, the possibly pathogenic antigen–IgG antibody complex having been successfully trapped in them by the " second line defence " anti-IgG antibodies. The free antigen–IgG complexes, untrapped by the latter, would then be the likely culprits causing tissue damage.

To conclude, whatever the role of the mixed cryoimmunoglobulins may be in the pathogenesis of various diseases, they seem to offer a multitude of possibilities for further studies, which may throw light upon the corresponding basic immunopathological mechanisms.

Immune complexes in leprosy

The pathogenic significance of immune complexes composed of mycobacterial antigens and the corresponding antibodies, as a probable major cause of tissue lesions in leprosy has been emphasized particularly by Almeida (1963a, 1963b, and personal communication).

Direct proof for the existence in leprosy of such pathogenic immune mechanisms, however, is lacking. The indirect evidence is only suggestive, inferior to that presented for analogous mechanisms in SLE (see above, pp. 796-797), and may be summarized as follows:

(1) The initial local lesions of the ENL reaction is characterized by vasculitis and polymorphonuclear leucocytic infiltration (Panel on Lepra Reaction, 1963), resembling the Arthus reaction (Turk, 1968), and the systemic pyrexial reactional phases with renal and joint involvement have common features with serum sickness.

(2) In reactional phases and following initiation of sulfone treatment, fresh sera of patients with lepromatous leprosy exhibit high anticomplementary activity, which is thermolabile (Almeida, 1963 and personal communication).[1] The haemolytic complement level (Eliasberg, 1911; Azevedo & Melo, 1966) and the level of $C'3_A$ (β_{1A}) is low (Bonomo et al., 1967).

(3) Free or excess mycobacterial antigen has been demonstrated in some leprosy sera (Rees et al., 1965) and polysaccharides, possibly of mycobacterial origin, in reactional states (Silva & Tuma, 1960).

(4) Leprosy sera caused sharp contractions of guinea-pig uterine horns under Schultz–Dale type experimental conditions. This property was heat-labile and was inhibited by anti-histamines (Castro, Silva & Castro, 1963).

[1] See also Almeida, J. O. (1963) *Work conference on the serology of leprosy*; unpublished Pan-American Health Organization working document RES.63.3.

(5) Renal lesions appeared in relation to the reactional phases of lepromatous leprosy; they began with proteinuria, which progressed eventually to renal failure (Granells, 1968). In a single patient with lepromatous leprosy, proteinuria appeared on the fourth day of the clinical ENL reaction (Turk, personal communication).

(6) Cryoglobulinaemia occurs in 50%–90% of patients with lepromatous leprosy (Matthews & Trautman, 1965; Bonomo & Dammacco, 1968 and personal communication). The cryoglobulins isolated from the sera of 2 patients with lepromatous leprosy were characterized by immunoelectrophoresis as IgM–IgG (Bonomo & Dammacco, personal communication).

Removal of cryoprecipitate abolished the rheumatoid factor, thyroid antibody and the VDRL activities of the parent lepromatous leprosy sera (Matthews & Trautman, 1965). Antinuclear factor activity disappeared from the lepromatous leprosy sera on storage at +4°C (Bonomo & Dammacco, personal communication).

The observations mentioned above are all compatible with the presence and activities of the postulated immune complexes. However, much more information than that available at the present time is needed to replace speculation by valid conclusions.

FUTURE LINES OF RESEARCH

From the immunopathological point of view, the same general rules apply to the study of leprosy as to that of the autoimmune diseases *sensu stricto*, say, SLE and rheumatoid arthritis. Intensive research on the latter has already resulted in most valuable information being assembled, strongly suggesting an essential pathogenic role for immune mechanisms in these diseases. The study of leprosy by modern immunological methods, however, has just begun but this disease, compared with the autoimmune diseases, offers the advantage of known etiology.

Studies to be carried out in the field of the immunopathology of leprosy, therefore, should aim at identifying the postulated participants (immunoglobulins, complement, mycobacterial antigens) in the possibly pathogenic immunological events at the sites of lesions, and also at tracing the pathways of reactions leading to the lesions of the target tissue. To this end, immunofluorescent techniques (and possibly other tracer-labelling techniques) should be the methods of choice.

Since *Myco. leprae* is the etiological agent of leprosy, more knowledge is greatly needed of its antigenic constituents, and also of those, possibly useful, cross-reacting antigens from other mycobacteria that are more easily propagated *in vitro* than the capriciously fastidious *Myco. leprae*. The studies by Ouchterlony and his co-workers (Norlin et al., 1966), being based on vast experience in the use of sophisticated analytical gel-diffusion techniques, may be regarded as most promising from this point of view.

The still-imperfectly understood life-cycle of the etiological agent of leprosy, manifesting itself as the acid-fast *Myco. leprae* bacillus when thriving in lepromatous human tissue, should also be given due consideration (Barksdale, personal communication).

Finally, the importance of mutual exchange of information extracted from the immunopathological study of the collagen diseases, on the one hand, and from that of leprosy, on the other, cannot, in the opinion of the writer, be stressed too much.

Detailed research plans do not fall within the scope of this general report but some suggestions for the partially overlapping main lines of future immunopathological study of leprosy are summarized below.

(1) Further extension of the studies of the AM (bursa-dependent) immunological aberrations, including cryoimmunoglobulinaemia, by the methods of modern immunology, as already successfully applied by Bonomo and his co-workers (Bonomo & Dammacco, 1967, 1968 and personal communication; Bonomo, Dammacco & Gillardi, 1968; Bonomo et al., 1963, 1965, 1967).

(2) Confirmation and extension of the recent challenging observations by Turk and his co-workers (Turk, 1968; Turk & Waters 1968; Turk & Willoughby, 1967) suggesting a genetically determined general depression of the CM (thymus-dependent) immune responses in lepromatous leprosy.

(3) Tracing of the reactants in the postulated immune complex-caused tissue damage in lepromatous leprosy.

(4) Isolation and characterization of soluble antigenic components from *Myco. leprae* and other, cross-reacting mycobacteria.

(5) Further development and analysis of the experimental model of *Myco. leprae*-caused disease in mice, with particular reference to the immunological aberrations possibly associated with it.

(6) Exploration of the life-cycle of *Myco. leprae*, e.g., search by tracer-labelling methods of *Myco. leprae*-specific defined antigens (see under (4) above) from tissue sections free of acid-fast bacilli.

(7) Despite frustrations associated with previous attempts at *in vitro* cultivation of *Myco. leprae*, the recent progress made in the methods of cultivation of other fastidious micro-organisms, e.g., viruses and mycoplasmas (Hoorn, 1966; Jansson & Wager, 1967), may encourage microbiologists to continued efforts at creating *in vitro* conditions acceptable to this true devotee among microbes of live human tissue.

RÉSUMÉ

LES ASPECTS IMMUNOLOGIQUES DE LA LÈPRE DANS LEURS RAPPORTS AVEC LES MALADIES AUTO-IMMUNES

La lèpre, en particulier sous sa forme lépromateuse, s'accompagne d'un très grand nombre d'anomalies immunologiques et ses aspects cliniques rappellent à maints égards ceux que l'on observe dans les maladies du collagène.

Plusieurs faits plaident en faveur d'une hyporéactivité, d'origine génétique, de l'immunité à support cellulaire chez les malades lépromateux. Outre l'absence de réaction à la lépromine, on observe chez ces patients toute une série de phénomènes explicables par la carence de cette immunité: sensibilité amoindrie vis-à-vis de divers antigènes lors des tests cutanés; affaiblissement de la capacité de transformation des lymphocytes; retard dans le rejet des homogreffes cutanées; présence, au niveau des ganglions lymphatiques, d'altérations histologiques comparables à celles qui succèdent chez le cobaye à l'administration de sérum anti-lymphocytes. Toutefois, on ne peut exclure complètement la possibilité que cet affaiblissement de l'immunité à support cellulaire résulte d'une action directe de l'infection par *Mycobacterium leprae*. Quant aux observations qui font état d'une stimulation générale des mécanismes immunitaires assurant la production des anticorps circulants, elles sont fragmentaires et doivent être interprétées avec prudence.

Après avoir brièvement rappelé les conceptions actuelles relatives à l'auto-immunité et les critères qui permettent de classer une affection dans le groupe des maladies auto-immunes, l'auteur énumère certaines anomalies (semblables à celles que l'on rencontre dans le lupus érythémateux disséminé et dans l'arthrite rhumatoïde) dont l'incidence est particulièrement élevée dans la lèpre lépromateuse: facteurs antinucléaire (30%) et rhumatoïde (50%), anticorps anti-thyréoglobuline (40%), cryoglobulines (95%), réactions sérologiques faussement positives pour la syphilis (70%), faible taux de complément (50%). Les auto-anticorps circulants ne font probablement pas preuve d'une nocivité directe pour l'hôte, mais les immun-complexes contenant des auto-anticorps peuvent avoir un effet pathogène.

On dispose d'un certain nombre de données expérimentales concernant le pouvoir phlogogène des complexes antigène-anticorps et leur rôle pathogène dans les maladies auto-immunes. Les cryo-immunoglobulines mixtes (formées de deux (IgG-IgM ou IgG-IgA) ou de trois immunoglobulines) mises en évidence dans le lupus érythémateux et dans certaines maladies microbienes à composantes auto-immunitaires (syphilis, néphrite et endocardite streptococciques, mononucléose infectieuse, pneumonie à *Mycoplasma pneumoniae*) représentent peut-être des immun-complexes circulants.

Dans la lèpre également, diverses observations suggèrent l'existence d'immun-complexes pathogènes: présence de cryoglobulines mixtes, analogies entre les lésions locales de l'érythème noueux lépreux et celles du phénomène d'Arthus, épisodes réactionnels généraux rappelant la maladie du sérum, activité anticomplémentaire transitoire, diminution de l'activité du complément et de sa teneur en certains constituants, présence d'antigènes mycobactériens dans le sérum, concomitance de l'apparition des lésions rénales et des épisodes réactionnels.

Les recherches sur l'immunopathologie de la lèpre doivent être conduites selon les mêmes principes que les études consacrées aux maladies auto-immunes *sensu stricto*. Les principaux secteurs vers lesquels cette recherche devrait être orientée sont succinctement évoqués.

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Annex

CLASSIFICATION OF ALLERGIC REACTIONS (modified from Gell & Coombs, 1963)

Type I (anaphylactic)

(a) General anaphylaxis.

(b) Local anaphylaxis (urticaria, Prausnitz–Kuestner reaction, asthma); mediated by IgE, complement not involved.

Type II (cytotoxic)

(a) Antigen of cell surface (transfusion reactions, haemolytic disease of newborn).

(b) Antigen attached to cell surface (apronal (Sedormid) purpura); mediated by IgG, IgM and IgA, complement usually involved.

Type III (Arthus)

(a) Arthus reaction and serum sickness.

(b) Toxic-complex syndromes (sensitivity to penicillin, systemic lupus erthematosus, polyarthritis, cryoimmunoglobulinaemia); mediated by Ag–Ab complexes involving precipitating IgG, IgM and IgA, complement involved.

Type IV (delayed)

(a) Tuberculin reaction and contact dermatitis.

(b) Homograft rejection; mediated by specifically modified mononuclear cells.

MARKERS FOR AUTOIMMUNE DISEASE (modified from Mackay & Burnet, 1960)

(1) Hypergammaglobulinaemia ($\geq$ = 2 g/100 ml).

(2) Autoantibodies (circulating or cell-fixed or both).

(3) Deposition of immunoglobulin or complement or both in affected tissue.

(4) Accumulation of lymphoid and plasma cells.

(5) Responsiveness to immunosuppressive drugs (steroids, mercaptopurine, etc.).

(6) Co-existence of disease with other lesions attributable to autoimmunity.

None of the markers is necessarily specific but occurring in combination they strongly suggest an autoimmune process.