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Etiological Factors in Delayed-type Hypersensitivity Reactions in Leprosy*

Why has leprosy, throughout its history and in so many cultures, been such a feared and mysterious disease? Probably one of the most important factors is the terrible disfigurement which can arise as a consequence of the infection. These deformities are almost entirely secondary to damage in peripheral nerves, resulting in misuse, disuse, and infection. Thus, nerve damage lies at the root of many of leprosy's problems, and such damage can occur across the entire spectrum of the disease, from the slow, insidious, "glove-and-stocking" anesthesia of lepromatous leprosy to the involvement of a single nerve at the tuberculoid pole. Between lie the borderline states, and here nerve damage can occur not only in a manner analogous to that at the poles but also as an acute phenomenon, with severe destruction occurring over the course of a few days, paralleled by skin involvement of an equivalent degree. These lepra reactions (type 1) are emergencies—unpredictable, progressive, possibly irreversible, yet potentially treatable—and are a serious problem in modern leprosy, particularly considering patients already under treatment.

In some ways, the problems of the type 1 lepra reaction epitomize those of leprosy itself, with infection, inflammation, and a disturbance of immunity leading to nerve damage and its sequelae. Here, however, the problem of defective immunity is given a new dimension, for we must consider not only "suppression" versus "activation" but also "useful" immunity versus "harmful" hypersensitivity, thought to provide the basis for these reactions.

Why study such reactions? One could argue this from the standpoint of the basic scientist or the clinician. From the immunological point of view, an understanding of the mechanisms of this form of hypersensitivity and its relationship to immunity and tolerance is central to the problems of leprosy and many other infectious diseases. For the clinician, such understanding might allow us to predict, treat, or effectively prevent these flare ups. I would like to integrate these points of view by discussing the type 1 reaction and its etiology at a number of levels, from the clinical and epidemiological through histological, cellular, and molecular. In order to do this, we need first to put the reactions into the context of the disease as a whole.

Reactions in context

The clinical states resulting from infection with *Mycobacterium leprae* form a spectrum which is paralleled closely at the histopathological and immunological levels.[1] In simplest terms, the lepromatous form is a disseminated disease, with large numbers of bacteria present in many areas of the body, associated with a weak cellular immune response. The tuberculoid form is a localized disease, with few bacteria present and lesions where the cell-mediated immune response is marked. Leprosy can also produce a subclinical disease, as shown in community surveys,[2] and if disease does appear, classically this is of the "indeterminate" type, usually leading to spontaneous healing. However, 25% of cases will go on to enter the spectrum described, frequently as borderline forms[3] lying between the two poles, and these are the states which particularly concern us. One of the most important features of these states is their instability, and type 1 reactions are thought to be a manifestation of this.

The analogy of a spectrum of host/parasite relationships is used in other diseases,

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[1] Ridley, D. S. Histological classification and immunological spectrum of leprosy. Bull. WHO **51** (1974) 451–465.

[2] Godal, T. and Negassi, K. Subclinical infection in leprosy. Br. Med. J. **3** (1973) 557–559.

[3] Strickland, N. H. The influence of immunosuppression and immunodeficiency on infections with leprosy and tuberculosis. Int. J. Lepr. **53** (1985) 86–100.

including tuberculosis (TB).[4] Here, another parallel may be found—the dissociation of resistance from hypersensitivity. It should be noted at the start that these terms refer to functions rather than mechanisms. "Resistance" in leprosy has been defined as the ability of the host to limit the bacterial load and to dispose of disintegrating bacillary materials.[5] Hypersensitivity may be thought of as an excessive reactivity to a sensitizing antigen, i.e., that which results in damage to host tissue. Coombs and Gell have divided this into four types (originally as a way of teaching medical students), representing different pathways of immune expressions.[6] Of relevance to this discussion is type IV, or delayed-type hypersensitivity (DTH), which is characterized by a mononuclear infiltrate, in contrast with the mast cell, complement activation, and immune complex mechanisms which predominate in the other three types.

The argument as to the importance of DTH in immunity in TB has been going on since the 1920s when Rich first suggested that the processes were separable (e.g., immunity can remain intact even after DTH has disappeared).[7] Mackaness and others have held the opposite view, noting that both phenomena tend to appear together, e.g., in vaccination and adoptive transfer experiments.[8,9] Youmans[7] has argued strongly that DTH and immunity in TB involve recognition of different antigens. For example, immunity is induced by polysaccharide or ribonucleotide antigens and DTH by protein components, a suggestion of great relevance for potential vaccines. The distinction is for some rather artificial, since in natural infections it is not possible to separate the two phenomena, the underlying cellular mechanisms are not yet established, and there is more than one type of DTH (see below).[10] However, the idea of "helpful" and "harmful" immune responses is still a useful one and a recurrent theme in the discussion of type 1 reactions.

The historical context

Ridley[1, 11, 12] was the first to clearly distinguish reversal reactions, differentiating these from downgrading reactions, erythema nodosum leprosum (ENL), and exacerbation nodules on clinical, histological and immunological grounds. This was an important step in clearing up a field which had been confused for many years (e.g., including "akuter schub," "acute infiltration reactions," and "reactional tuberculoid" states). Essentially, we are left with two main forms of reactional state: type 1 and type 2 (not to be confused with types I–IV of hypersensitivity).

Type 2 lepra reactions or ENL are not under discussion here, except to distinguish them in that they occur mainly in lepromatous leprosy (rarely in borderline lepromatous), commonly rather later in treatment, and involve more systemic symptoms.[12] The underlying mechanisms are humoral, and ENL has been described as a clinical manifestation of the Arthus phenomenon.[13] In common with the type 1 reaction are a number of precipitating factors (intercurrent infection, physical and mental stress, vaccination, pregnancy, and chemotherapy),[12] and as with type 1 reactions, the mechanisms by which these act are uncertain. The process of defining these reactions is still continuing, as they are char-

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[4] Lenzini, L., Rottoli, P. and Rottoli, L. The spectrum of human tuberculosis. Clin. Exp. Immunol. **27** (1977) 230–237.

[5] Bjune, G., Barnetson, R. St.C., Ridley, D. S. and Kronvall, G. Lymphocyte transformation test in leprosy; correlation of response with inflammation of lesions. Clin. Exp. Immunol. **25** (1976) 85–94.

[6] *International Dictionary of Medicine and Biology*. New York: John Wiley and Sons, 1986.

[7] Youmans, G. P. Relation between delayed hypersensitivity and immunity in tuberculosis. Am. Rev. Respir. Dis. **111** (1975) 109–118.

[8] Mackaness, G. B. The immunology of antituberculous immunity. Am. Rev. Respir. Dis. **97** (1968) 337–344.

[9] Lefford, M. J. Delayed hypersensitivity and immunity in tuberculosis. Am. Rev. Respir. Dis. **111** (1975) 243–246.

[10] Salvin, S. B. and Neta, R. A possible relationship between delayed hypersensitivity and cell-mediated immunity. Am. Rev. Respir. Dis. **111** (1975) 373–377.

[11] Ridley, D. S. Hypersensitivity and immune reactions and classification. Lepr. Rev. **47** (1976) 171–174.

[12] Ridley, D. S. Reactions in leprosy. Lepr. Rev. **40** (1969) 77–81.

[13] Wemambu, S. N., Turk, J. L., Waters, M. F. R. and Rees, R. J. W. Erythema nodosum leprosum—a clinical manifestation of the Arthus phenomenon. Lancet **2** (1969) 933–935.

acterized at an increasingly more detailed level.

Evidence from the clinical/epidemiological level

Type 1 reactions can be divided into upgrading and downgrading, although the status of the latter is still under dispute.[14] Here we are concerned with the upgrading or reversal reaction, associated with an increase in cell-mediated immunity (CMI) and a decrease in bacterial load, i.e., a shift across the leprosy spectrum toward the tuberculoid end. The commonest shifts which occur are in the borderline forms: borderline lepromatous (BL) to mid-borderline (BB), BB to borderline tuberculoid (BT), BB to subpolar tuberculoid (TTs), while subpolar lepromatous (LLs) to BL is rarer. Ridley suggests that TTs patients may undergo reactions resulting in rapid healing, but certainly the most severe reactions occur in the middle of the leprosy spectrum, the states nearer the poles being more stable.[11]

The features of an upgrading reaction involve inflammation and edema of preexisting lesions in skin and nerves, although new lesions may appear in previously clear areas (invisible patches[15]). The warm, erythematous skin lesions may undergo necrosis as the reaction develops. These in themselves are serious for the patient, but far more serious from the prognostic point of view are the reactions within the nerves. Inflammation and edema within the nerve sheath lead to functional impairment and nerve pain (neuritis). The latter may be severe (although characteristically very severe pain is associated with ENL, where the functional impairment may be less prominent[15]).

Sensorimotor impairment lies at the heart of the problem, for unlike the polar forms, this may lead to an acute paralysis, which, despite treatment, may progress through fibrosis. The nerves most at risk are the common peroneal (lateral popliteal), the ulnar and the facial, leading to foot drop, claw hand and facial palsy, respectively, the latter with a particularly poor chance of recovery.

Barnetson and colleagues[16] distinguish three types of reaction on clinical grounds—skin reactions, nerve reactions (for some reason more common in men[17]), and a mixed type. The patients in different groups demonstrate different antigen sensitivities (see below), underlining the fact that careful clinical observation can yield clues as to the cellular mechanisms involved.

Systemic illness may also occur and, indeed, a prodromal stage has been noted, involving generalized aches and pains, paresthesiae and malaise.[18] Edema may occur in the hands, feet or face; tenderness of one foot may be a first warning sign. Edema itself is an important part of the reversal reaction, and could contribute to tissue damage through pressure effects. One study of the Mantoux test has demonstrated that whether such a reaction is clinically positive or negative depends to a great extent on the presence of tissue edema, regardless of the cellular reaction.[19] Other features of systemic illness include a low-grade fever and anorexia.[17] The fever may arise as a consequence of the reaction or could, perhaps, be related to precipitating it, since small rises in temperature may be associated with a marked enhancement of cell-mediated immune function (up to 20-fold).[20] Thus, the fever could act as an important part of a positive feedback mechanism in this sudden surge of immune activity.

Epidemiology. Few studies have been done to study these reactions from a population or community perspective, and there are obvious logistic problems with this, in

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[16] Barnetson, R. St.C., Bjune, G., Pearson, J. M. H. and Kronvall, G. Cell-mediated immunity and humoral immunity in reversal reactions. Int. J. Lepr. **44** (1976) 267–273.

[17] Hastings, R. C., ed. *Leprosy*. Edinburgh: Churchill Livingstone, 1985.

[18] Barnetson, R. St.C., Pearson, J. M. H. and Rees, R. J. W. Evidence for the prevention of borderline leprosy reactions by dapsone. Lancet **2** (1976) 1171–1172.

[19] Black, S., Humphrey, J. H. and Niven, J. S. Inhibition of the Mantoux reaction by direct suggestion under hypnosis. Br. Med. J. **5346** (1963) 1649–1652.

[20] Atkins, E. Fever—new perspectives on an old phenomenon. N. Engl. J. Med. **308** (1983) 958–969.

addition to the difficulty of consistently accurate diagnosis. One common observation is that the period of greatest risk is the first 6 months or 1 year of treatment, although reversal reactions may occur in untreated patients, and patients may initially present in reaction.

The view is commonly held, therefore, that chemotherapy is directly responsible for this increased risk, particularly dapsone therapy. This will be discussed further below, but it should be pointed out that it is not a universally held opinion; for example, Barnetson and colleagues[18] provide evidence that high doses of dapsone may provide a protective effect. Other regimens claimed to have a protective effect include clofazimine therapy, and Pfaltzgraff and colleagues have provided evidence on this point.[21] However, reactions can also occur after clofazimine therapy, and the issue at a clinical level is far from clear.[22]

Important precipitants other than therapy include intercurrent infection (especially TB), stress, vaccination (including BCG), and pregnancy.[17, 23] With regard to the latter, the time of greatest risk is the puerperium, when there is a return to normal of CMI from its depressed state.[24] Stress is also an immunostimulant, and two pathways may be of importance:[25] "hardwiring" to lymphoid tissue, spleen, etc., from the nervous system, and humoral links through a remarkable number of shared chemical transmitters (e.g., endorphins, substance P) which may act in both directions, making the immune system a "mobile brain." This field of psychoimmunology is still in its infancy. A disturbance of immune equilibrium may underlie the action of all these precipitants, although evidence for their effect remains largely confined to the clinical (in some cases almost anecdotal) level.

Histology

The natural course of upgrading reactions has been rarely described because of the necessity for immediate suppression. Ridley and Radia[26] had the opportunity to study a number of cases and identify changes at a histological level. The course of the reaction can be divided into four stages (early, acute, late, and resolving), although this is variable. The first changes include an influx of mononuclear cells but the prominent feature of the acute stage is edema, leading to distortion of the surrounding tissues. Fibroblast proliferation and elastosis may be accompanied by necrosis, and later the lesion assumes more tuberculoid features with well-defined clusters of cells, including epithelioid and giant cells. Ultimately, a resolving lesion is left in which fibroblastic activity is prominent. Downgrading reactions, in contrast, show initial edema but do not go on to tuberculoid maturation.

Parallel changes take place in reactive lymph nodes, although to a much less marked degree, the nodes tending to assume the appearance of the upgraded state. Changes throughout the dermis (i.e., including unaffected areas) have been noted, and it is suggested that the reaction may be of a more generalized type, perhaps involving a certain auto-immune component.[27] In this sense, the description of the reaction as "tissue panic" is very apt.[28]

Also visible in histological sections are lipids present within macrophages.[26] Fat is normally found in lepromatous "foamy" macrophages but not in tuberculoid epithelioid cells. Campos, however, found that lipids were commonly found in macrophage-derived cells in lesions undergoing reaction.[29]

Comparison with DTH. The entire reaction has been compared with the DTH of a tuberculin (or lepromin) reaction taking

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[22] Mahapatra, S. B. and Ramu, G. Transformation from lepromatous to borderline leprosy with clofazimine therapy. Lepr. India **48** (1976) 172–176.

[23] Wade, H. W. BCG-induced activations. Int. J. Lepr. **28** (1960) 179–181.

[24] Rose, P. and McDougall, A. C. Adverse reactions following pregnancy in patients with borderline (dimorphous) leprosy. Lepr. Rev. **46** (1975) 109–114.

[25] Blalock, J. R. and Smith, E. M. Psychoimmunology. Immunol. Today **7** (1986) 115–117.

[26] Ridley, D. S. and Radia, K. B. Histological course of reactions in borderline leprosy and their outcome. Int. J. Lepr. **49** (1981) 383–392.

[27] Ridley, D. S. and Wise, M. J. Reactions of the dermis in leprosy. Int. J. Lepr. **32** (1976) 24–36.

[28] Cochrane, R. E. and Davey, T. F. *Leprosy in Theory and Practice.* Bristol: John Wright and Sons, 1964.

[29] Campos R. de C., J. Lipoids in reactional tuberculoid leprosy granulomata, their diagnostic value. Int. J. Lepr. **18** (1950) 155–160.

place within multiple lesions. Indeed, these have similar histological characteristics,[30] with an influx of monocytes and lymphocytes, early disturbance of collagen fibers through edema, and subsequent giant cell formation. It should be noted, however, that there is more than one type of DTH reaction. For example, the cutaneous basophil response or Jones-Mote reaction bears very little relation to the above picture.[31] Also mast cells have been implicated in certain types of DTH,[32] although such activity has not been described in reversal reactions.

It had been suggested from experiments with resistant and susceptible mice that it is the kinetics of the DTH reaction which correlates with immunity rather than the degree.[33] By careful analysis of this, Rook and Stanford have been able to separate DTH reactions into three types: the Jones-Mote, a "Listeria type," and a "Koch type."[34] The Listeria type occurs early, 10–12 days after sensitization, is nonnecrotic, and has a shorter time-course. The classical Koch type occurs after 4–6 weeks, with a longer time-course, and is associated with necrosis. For instance, C57BL mice are resistant to *M. lepraemurium* given subcutaneously, and produce a strong Koch-type response. However, for intravenous infection, this response appears to be inappropriate, and they are highly susceptible.

In simplistic terms, the Listeria-type response may be associated with intracellular killing and the Koch-type with extracellular killing, and it is of interest that cytotoxic T cells are seen in the latter but not in the former.[35] It may prove helpful to view the type 1 reaction from this perspective.

Summary of histological evidence. The histological evidence points strongly to a flare up of DTH—given the presence of necrosis this seems to be similar to the Koch type. The early appearance of edema together with the more generalized tissue reactions imply that the situation may not be quite so straightforward. In addition, the reactions in nerves, which are of the greatest clinical significance, are the hardest to study. Barnetson and colleagues[16] found that, in the skin, reactions often took place within dermal nerves.

The presence of fat is of some interest given the potential role of lipid oxidation products in tissue damage,[36] for example, in atherosclerosis.[37] They may also have more subtle effects. Presentation of antigen in conjugation with lipids is known to predispose to DTH,[38] and Skinsnes has proposed that defects in lipid handling by macrophages may be of central importance in leprosy.[39]

Finally, the fact that the reaction has such a clearly defined course at the histological level should lead us to consider those factors which serve to terminate the process, as well as those which precipitate and maintain it. Manipulation of these may be of therapeutic importance, although a fuller understanding requires evidence from the cellular and molecular levels.

Cellular and molecular evidence

The use of monoclonal antibody technology has allowed the dissection of the cellular components of these reactions with a good deal of precision. In addition, a comparison of the function of T cells in reactive and nonreactive lesions can be made by use of the lymphocyte transformation test

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[36] Fantone, P. and Ward, J. The role of oxidized free radicals in leukocyte dependent inflammatory reactions. Am. J. Pathol. **107** (1982) 397–401.

[37] Mitchinson, M. J. Macrophages, oxidized lipids and atherosclerosis. Med. Hypoth. **12** (1983) 171–178.

[38] Dailey, M. O. and Hunter, R. L. The role of lipid in the induction of hapten-specific delayed hypersensitivity and contact specificity. J. Immumnol. **112** (1974) 1526–1534.

[39] Skinsnes, O. K. Leprosy and the lipidoses. Int. J. Lepr. **44** (1976) 482–484.

(LTT), an *in vitro* assay of T-cell responsiveness. Bjune and colleagues[5] found that the LTT correlates well with the degree of hypersensitivity, but rather poorly with the degree of immunity or "resistance," i.e., the position on the Ridley/Jopling scale. This brings us back to the dissociation of DTH and immunity—whether the results of the LTT reflect a qualitative change in immune function (that is, reacting to a different antigen) or a quantitative imbalance between DTH and other immune activity is an important question.

Studies have also been performed to distinguish the various T-cell subsets involved in the reactions. The ratios of T4+ (helper/inducer phenotype) to T8+(suppressor/cytotoxic phenotype) and their distribution have been compared to that in tuberculin and lepromin reactions.[30, 40, 41] In the initial (borderline) state, about 40% of granuloma cells are T lymphocytes, with a T4+:T8+ ratio of 2:1 and no tendency for subset segregation within the lesion. In reaction, a number of changes occur including, in a minority, a redistribution of the suppressor/cytotoxic subgroup to the periphery of the lesion, thus assuming a more tuberculoid appearance. The majority of the lesions, however, show no "mantle zone" redistribution of T8+ cells. The total number of T cells within the lesion rises to 50%, and the T4:T8+ ratio drops to 1.5:1 (a difference from nonreactive borderline which does not reach statistical significance).

In DTH reactions, as in the classical tuberculin response, there is lymphocytic influx at a helper/inducer:suppressor/cytotoxic ratio of 2:1, the same as is present in the circulation, thus implying there is no preferential emigration. T4+ cells are, however, more prominent within the epidermis where they are found in close association with Langerhans' cells. Evidence from mouse models suggests that the helper T cell is the prime mediator of DTH, and it is hypothesized that suppressor T cells may be responsible for local control of the reaction.[40] Alternatively, T8+ cytotoxic cells may be important effector cells (which might explain why it is possible to have a flare up of DTH without a significant change in the T4:T8 ratio), although currently it is not possible to accurately distinguish cytotoxic from suppressor subtypes immunohistochemically.

Other T-cell subsets include "contrasuppressor" cells which are thought to inhibit suppressor mechanisms, thus releasing local expression of immunity.[42] Such cells are associated with particular sites, including the spleen, gut, and skin, and represent a hypothetical mechanism whereby suppression could be temporarily "bypassed" in borderline patients.

Antigen presentation. Another important cell isolated by these techniques is the T6+ Langerhans' cell found in normal skin and in the regions encircling the granuloma.[30] These cells are known to be potent antigen-presenting cells,[43] and it is suggested that this function arises as a result of the high levels of class II major histocompatibility molecules (HLA-DR), which act as restriction elements for helper T cells, and their ability to secrete interleukin-1 (IL-1). This molecule is of central importance in immune activation as well as having major effects on inflammation and fever. The position of these cells in upgrading lesions implies that they are playing some role in contact with participating lymphocytes, not seen in normal borderline leprosy. They may be acting as antigen-presenting cells *in situ* or, as part of the "skin immune system" drain through lymphatics, undergoing maturational changes, to eventually serve as antigen-presenting cells in the local lymph node.[44]

Other notable cellular changes include a generalized induction of HLA-DR on kera-

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[43] Britz, J. S., Askenase, P. W., Ptak, W., Steinman, R. M. and Gershon, R. K. Specialized antigen-presenting cells. Splenic dendritic cells and peritoneal-exudate cells induced by mycobacteria activate effector T cells that are resistant to suppression. J. Exp. Med. **155** (1982) 1344–1356.

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tinocytes. It is also known that keratinocytes can act as antigen-presenting cells, and, in addition, can secrete IL-1.[45] Activated endothelial cells in regions of DTH may also present antigen, and are known to secrete a molecule (ICAM-1) involved in lymphocyte diapedesis.[46]

How may these processes be paralleled in neural tissue? Of relevance here is the finding that in the rare disease chronic inflammatory demyelinating polyneuritis, HLA-DR may be induced on Schwann cells which may, therefore, act as antigen-presenting cells.[47] Interferon-γ (IF-γ) *in vitro* may effect this induction, and it is proposed that this represents a simple quantitative change and, indeed, that quantitative variation in HLA-DR may play a central role in immunoregulation.[48]

Molecular mediators. The role of IF-γ as an immunomodulator is highlighted by the work of Nathan and colleagues, who have found that local injection of recombinant IF-γ into lepromatous lesions leads to a reaction *in situ* indistinguishable from DTH.[49] The clinical/histological criteria as discussed are all well demonstrated and, in addition, an increased killing ability of monocytes and a decreased bacterial load in the lesions are seen. In other words, there is an increase in local CMI without a generalized reversal reaction. These similarities point to IF-γ having some role in upgrading reactions, and the authors suggest that in their experiment it acts through recruitment of monocytes and activation of macrophages. It is hypothesized that IF-γ released by activated T cells may induce many of the manifestations of DTH and CMI, and it may even be possible to enhance only the CMI or microbial killing functions without increasing the DTH or tissue destructive function. This may be optimistic, for IF-γ is an important "priming" agent for both intra- and extra-cellular killing by macrophages. Macrophages are activated by sequential "priming" and "triggering" steps.[50] In lepromatous lesions, macrophages primed in this manner may be triggered by the large numbers of bacteria present to an intracellular killing function, while the different environment of the borderline lesion may favor triggering to extracellular activity and, hence, tissue destruction. By analogy, Youmans and colleagues have suggested that in TB separate lymphokines may be involved in DTH and immunity,[7] and elucidation of these is a subject for future research.

Other mediators include those secreted by the macrophages themselves. These are not only factors which activate lymphocytes and antigen-presenting cells, but also those which suppress them. These include prostaglandins[51] and, possibly, interferons.[52] Gupta and colleagues, using cell sorting techniques in a murine granuloma model, have isolated a small suppressor subpopulation.[53, 54] It is known that pro-

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duction of these suppressor factors may be modifiable (for example, diets rich in polyunsaturated fatty acids induce suppressor macrophage activity, possibly through changes in prostaglandin synthesis[51]). These factors may be relevant to the precipating effects of dapsone, and may even provide clues to a suitable therapeutic approach.

Biochemical aspects of chemotherapy. Chemotherapy is thought to be an important etiological factor in reversal reactions, but is there any evidence for this at the biochemical level? Drugs may potentially affect immune responses in two ways: a) by action on the bacteria themselves, thus changing the antigenic load (see below), or b) as will concern us here, through intrinsic immunostimulatory activity.[55]

Dapsone is thought to possess immunostimulatory properties, although the mechanisms for these are not clear. It is suggested that dapsone may contribute to reversal reactions by enhancing lymphocyte responsiveness,[55] and it may do so by inhibiting production of suppressor prostaglandins through its antioxidant activity. During chemotherapy, with recovery of CMI, T cells would, under normal circumstances, become more responsive to prostaglandin-mediated inhibition. If dapsone suppressed this, susceptibility to reversal reactions would be increased, but there is no evidence as yet for this hypothesis. *In vivo* experiments have shown that lymphocyte responsiveness is potentiated by antioxidant drugs, which may also enhance DTH to mycobacterial proteins in rats.[56] This last effect may also be achieved by modulation of dietary lipids—perhaps of some relevance to the use of chaulmoogra oil, an ancient remedy for leprosy, supposedly of benefit through its content of fats of high melting point.[57]

Dapsone also has an anti-inflammatory action, as is demonstrated by its effectiveness in a variety of dermatological and inflammatory disorders, e.g., relapsing polychondritis.[58] High doses of dapsone have also been reported to show a protective effect in reversal reactions,[18] and this may be due to a quantitative balance of its immunostimulatory, antimicrobial, and anti-inflammatory actions. The ability of the drug to scavenge oxidative intermediates is again thought to be important,[59] and it is interesting that the mutually antagonistic effects of clofazimine and dapsone at the clinical level are mirrored biochemically. Prostaglandin E_2 is known to suppress activated macrophages.[60] Anderson provides evidence that its production is enhanced by clofazimine but inhibited by dapsone,[61] suggesting future possibilities for prevention.

A further anti-inflammatory action of dapsone may be on the degradative processes of phagocytes, where it is thought to inhibit lysosomal activity.[58] Lysosomes may also be involved in antigen processing.[62] A change in the mechanism of antigen processing could have significant effects on immune function, since it is known that the exact form in which an antigen is presented is crucial to the type of response which develops. Dapsone has been described as "lysosomotrophic,"[58] and such agents are used

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experimentally to manipulate the immune response.[63]

Integration

So far, I have discussed evidence from a variety of approaches as to mechanisms which may be involved in reversal reactions. I shall now attempt to synthesize some of this information into three broad etiological schemes: a) qualitative antigen changes, b) quantitative antigen changes, and c) changes in the underlying immune response.

Qualitative antigen changes. The study by Barnetson and colleagues[16] provides evidence that the antigens to which those in reaction are responding are distinct, even within different reacting subgroups. It is suggested that in nerve reactions cytoplasmic antigens are released which had previously been hidden within Schwann cells, while in the skin there is an equivalent release of surface antigen from dermal nerves. The triggers for this remain obscure, and the possibility of coincidental exposure to other mycobacterial antigens was not borne out in this experiment.

As discussed, there is evidence in TB that the antigens which determine immunity and DTH are distinct.[7] A release of "hypersensitivity-inducing" antigens from dead bacilli as a result of chemotherapy or an underlying change in immunity could, therefore, be hypothesized in reversal reactions. If so, discovery of which particular antigens are involved would be an interesting area for research.

One alternative explanation for the results of Barnetson and colleagues relates to the sonication technique used to separate "surface" and "cytoplasmic" antigens. Sonication is known to affect the physical properties of proteins, leading to aggregation and insolubilization,[64] and may therefore have an effect on the presentation of antigens in this experiment, given the particular importance of surface properties in this function.

Quantitative antigen changes. Ridley[11] suggests that just as important as any change in antigen type may be a change in quantity, leading to an imbalance of immunity and hypersensitivity, as had been postulated nearly 80 years ago in TB.[65] He points out that it is very unlikely that bacterial antigens will never have been previously encountered, given the bacillary load of borderline patients. As the load is reduced, it may be that pre-existing antigens are "noticed" by the immune system, leading to the hypersensitivity response. This immunity/hypersensitivity balance is possibly of fundamental importance in understanding the host/parasite relationship in leprosy, and may be applicable even in the polar forms.

Other aspects of quantitative changes include a redistribution of bacterial antigens, e.g., via the bloodstream to the spleen. Route of antigen exposure can be important in determining responsiveness,[66] and the facts that circulating lymphocytes show increased activity[17] and that the illness is systemic could be clues in this direction. In addition, it should be remembered that T-helper cells recognize not one antigen but a combination of surface antigen and HLA-DR. An increase in HLA-DR expression (which is depressed in the chronic state[67]) could lead to an increase in "visible" antigen. This could occur either on new cells, e.g., Schwann cells, or through further stimulation of cells already HLA-DR positive, either to maintain the reaction (as a cascade phenomenon) or in initiation.

Underlying change in immunity. That possible etiological factors may act through effects on the host's underlying immune activity has already been suggested: how might this precipitate a reversal reaction?

Immune equilibrium. The balances between activation and suppression, hypersensitivity and immunity in borderline states are fine ones, and only small changes may be required to push the patient over the

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leprosy "precipice." One way of modeling this may be by use of catastrophe theory,[68, 69] a mathematical system developed to describe sudden changes in state. As long as a balance between hypersensitivity and immunity is maintained a smooth course is pursued, but imbalance leads to catastrophic changes such as reversal reactions.

Bypass: Afferent limb. Immunostimulation not only applies to the lymphocytes themselves but also to their means of activation. An intercurrent viral infection, for example, by inducing interferon production, could allow activation of otherwise quiescent antigen-presenting cells. An influx of new lymphocytes into the region would cause further stimulation of these cells and, hence, the crescendo of a reversal reaction. In particular, antigen presentation by previously inert cells such as keratinocytes and Schwann cells could be important.

Bypass: Efferent limb. Suppressor mechanisms may also be bypassed by direct activation of effector cells, or involvement of new cell types. The role of the cytotoxic T cell and even natural killer (NK) cells in these lesions has yet to be determined. (For example, NK cells are activated by BCG[70] and, in turn, activate macrophages through secretion of IFN-γ.[71]

Probably all of these features, a change in antigen type, load, and underlying responsiveness, have some role to play. The cellular reactions occurring in even the most simple experimental model of DTH are poorly understood. No wonder that the mechanisms of reversal reactions, occurring as they do suddenly, unexpectedly and under difficult medical conditions, are still a mystery.

Future possibilites

How may we proceed in order to unravel this mystery? In classical epidemiological fashion, the procedure to be followed is:

Biological expectation
Case reports
↓
Cross-sectional study
↓
Case-control study
↓
Cohort study
↓
Randomized intervention study

With reversal reactions, we are still in the early stages. Case-control and cohort studies need to be carrried out to define risk factors more rigorously. This is obviously a difficult task, given the situations in which leprosy is prevalent and the difficulty of accurate diagnosis of reactions. However, a controlled statistical approach in those areas where it is possible would be of immense benefit, and might even give us the ability to predict those patients who are at risk at the start of treatment.

Further work on the detailed biochemistry of dapsone and other agents may not be as rewarding as analysis of these drugs in a clinical setting. If high-dose dapsone does provide a degree of protection then trials could be more widely extended in patients at risk. Alternative anti-inflammatory medication may be useful as a prophylactic measure (particularly, perhaps, relating to prostaglandin synthesis).

While vaccination programs using *M. leprae* are going ahead in a number of centers to provide increased immunity, it is also of interest to note that certain methods of inoculation can lead to induction of tolerance. In the mouse, DTH can be suppressed by inoculating intravenously, subcutaneously in high doses, or in the presence of ultraviolet (UV) light (UV in solar doses can induce suppressor activity[72]). If, indeed, DTH in reversal reactions is directed against a particular antigen or set of antigens, it may be possible to vaccinate against these reactions by use of such a suppressive protocol. This would require elucidation and

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subsequent purification of the antigens responsible, but the application of DNA technology to such problems has already begun.[73]

There is now the potential to understand and prevent these reactions. While leprosy remains a problem of the world's poor countries, we in the Northern Hemisphere, where technology and scientific support are available, have the opportunity to tackle many aspects of the disease. This opportunity must be seized.

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