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# Anergy : depressed delayed hypersensitivity in Hodgkin's Disease

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# ANERGY - DEPRESSED DELAYED HYPERSENSITIVITY IN HODGKIN'S DISEASE

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## Introduction

One of the several immunological abnormalities developing in Hodgkin's disease is that of anergy. In order to attempt an understanding of anergy, it's manifestations, fluctuations, and possible etiology, it is first necessary to review delayed hypersensitivity.

## Delayed Hypersensitivity

### Immune reactivity - delayed type

Immune reactivity is characteristically divided into immediate or delayed type. The distinction is important since the two are probably mediated by different mechanisms. The immediate hypersensitivity reaction is mediated by antibody, and the delayed hypersensitivity reaction by cells (1). Two prototypes are recognized in delayed hypersensitivity, that of infective, with Tuberculosis as the classic example, and contact sensitivity which includes a variety of substances ranging from chemicals to oily resins of plants as allergens. Investigations of infective sensitivity reveal the protein moiety of bacteria and virus, and the polysaccharide moiety of fungus to be the sensitizing constituents (23). In contact sensitivity the sensitizing constituents were found to be those which were able to combine with proteins of the epidermis (8). Arguments favoring cell mediation of delayed hypersensitivity vs. blood protein

mediation are cited by Chase (9). First, he demonstrated that delayed reactivity could not be transfered from a sensitive to a normal subject by means of serum, but it can be passed by cells obtained from lymphoid tissue, peritoneal exudate or peripheral blood. These cells are lymphoid in origin as indicated in successful transferes, and small lymphocytes are probably most active as evidenced by their accumulations in developing tuberculin reactions. Tritiated thymidine labeled cell studies have substantiated this finding (20). Secondly, it is noted in clinical states such as hypogammaglobulinemia that antibody production is virtually indetectable while delayed hypersensitivity is intact. Conversely, in Hodgkin's disease (predominantly early Hodgkin's) there is no detectable antibody deficiency, yet there is depressed delayed hypersensitivity.

Antigen distribution

In a recent paper by Nossal and Abbot (21),isotope labeled and electron microscope studies of antigens IN VIVO revealed a localization of antigen in the draining lymph node following subcutaneous injection. More specifically, the lymph node medullary macrophages seemed to avidly phagocytize the antigens. Heavy labelling was observed over the lymphoid follicles in the cortex of the nodes. Electron microscope findings in close study of the lymphoid follicles showed the antigen to appear

to be predominantly associated with cell membranes of the fine reticular cell processes. Small lymphocytes were sometimes noted to cluster around heavy antigen depot sites and occasionally small amounts of label could be seen in the nucleus of small lymphocytes. From these observations, one "may postulate that antigen is released from the reticular cell depot, penetrates the lymphocyte nucleus and plays an inductive role". With antigen localized in follicles, it is in effect captured and retained on the surface of a complex network of fine cell processes. This suggests that antigen may persist, undenatured and highly accessable to adjacent lymphocytes. Rats deprived of small lymphocytes by chronic thoracic duct drainage fail to respond to a primary antigenic stimulus.

Tissue response in delayed hypersensitivity

Aisenberg (1) has established ground rules in the study of delayed allergy, limiting the phenomenon to skin reactions and the slow evolution of such reactions over a period of 24 to 48 or more hours after application of the antigen. This criterion is useful in differentiating delayed responses from antibody mediated Arthus skin reactions as well as eliminating Jones-Mote reactivity, an antigen-antibody complex, as a possible inducer of true delayed hypersensitivity. Subsequent studies by Raffel and Newel (23) proved that the antigen alone as a soluble protein

administered by any route fulfilled some of the criterion for delayed Jones-Mote reactivity, but found this to be a transient phase that was subsequently replaced by a typical Arthus responsiveness and circulating antibodies.

Skin testing for delayed hypersensitivity

Delayed hypersensitivity is assessed by the use of skin tests employing derivatives of allergens that evoke the response. In years past delayed hypersensitivity has largely been evaluated by tuberculin skin tests; however with fewer people in the general populace showing no reactivity to this test it has been necessary to use other allergens, some determined by locale where possibility of exposure is greatest. Some of the delayed type allergens that have been and are being used include Purified Tuberculoprotein (PPD), Mumps skin test, Candida albicans, Trichophyton, Coccidiodes immitis and Histoplasma capsulatum. Another technique is that of induced sensitization, which eliminates the problem of people who have possibly never been exposed to the above allergens. These include use of the BCG and dinitrochlorobenzene sensitization. The use of dinitrochlorobenzene has shown almost universal positive results in normal patients and the technique (2) is such that implementation of its use as a control in skin testing as well as specific studies in anergy should be more widely considered and accepted. Positive reactions

occur three weeks following primary sensitization by applying a weak solution of DNCB that simulates a delayed response in most respects, save for a vesicle formation that occurs in many instances. Another interesting technique is that of the use of depot tuberculin (7). This is the use of tuberculin emulsified in lanolin, then blended with paraffin oil, that can detect reactivity in persons with BCG vaccination.

## Anergy

### Definition and tests for Anergy

Having established some basic concept of what is known of delayed hypersensitivity a consideration of anergy or depression of delayed hypersensitivity is in order. Aisenberg (1) defines anergy as an "immunological defect occurring early in active Hodgkin's disease characterized by depresssion of delayed hypersensitivity and assessed by negative reaction to a battery of skin allergens". The basic defect appears to be peripheral i.e. a manifestation of abnormal lymphocyte function. This is in apposition to a central failure. Depression of delayed hypersensitivity in the face of essentially normal antibody formation and recovery of skin allergy during disease remission imply an intact central mechanism. Abnormalities of Hodgkin's lymphocytes IN VITRO and after transfer studies support this contention. There seems to be general agreement among authors

that the lymphocyte is the basic defective element in anergy, but there is conjecture about it being secondarily affected by a plasma factor (7).

Occurrence and variations of Anergy in Hodgkin's and other diseases

The occurrence of anergy in various disease states has in the past offered hope of perhaps helping to establish an etiology of Hodgkin's disease. The cutaneous exanthums, in particular measles, have been noted to evoke an anergic state arousing thoughts of possible viral etiology. Neoplastic diseases where anergy has been noted include Leukemia, Carcinoma, and the other lymphomas. Sarcoidosis is also commonly known to induce anergy. Interestingly Hodgkin's anergy has been noted to be somewhat unique in that it occurs very early in the active disease while the patient is in a good clinical state whereas in Carcinoma, leukemia and the other lymphomas anergy occurs late and when the patient is in a poor, nearly terminal clinical state (cachetic anergy) with leukocyte and immunoprotein abnormalities. Conversely, in Hodgkin's, anergy is noted early when the lymphoid cell is present in normal quantities and there are no demonstrable antibody deficiencies (16). Sarcoidosis anergy on the other hand, precipitated by a generalized granulomatous process can be reversed by the "depot tuberculin" technique already mentioned as well as cellular transfer of immunocompetant cells (9,17,18).

Attempts of reversing anergic states in Hodgkin's by these methods however have failed. Likening of failure to exhibit tuberculin reactivity during measles or cutaneous exanthums has drawn criticism on the basis of altered skin in these viral infections. It has been noted that this failure has been induced by measles vaccine as well (28).

Fluctuations in the Anergic State

An observation of anergy development (24) by use of the tuberculin test in Hodgkin's disease in four instances, revealed a time of 2,3,5 and 8 months. Unfortunately, a survival time has not been established which might offer a form of prognosis. However, because of the decreased incidence of tuberculin reactivity and few instances of chance tuberculin skin testing on individuals eventually affected with Hodgkin's disease, it would appear to leave much to be desired as any kind of useful prognostic tool. Observed fluctuations in the anergic state however, do seem to reflect the activity of the disease. A reversion to tuberculin reactivity was correlated with clinical improvement, whereas continued anergy represented no change or a worsening in the clinical state (27). Aisenberg (3) states, "A recovery from anergy should represent a reversion to normal metabolic pathways within the potentially immunocompetent cells, or within other cell types in which metabolic products might arise that would affect the immunocompetency". Fluctuations

noted in anergy to the contact allergen dinitrochlorobenzene showed conversions to positive reactions by (a) irradiation therapy, (b) uniformly in patients where the disease was inactive for a period greater than two years, and(c) occasionally in patients where the disease was inactive for a period of from 2 to 24 months. Reconversion to tuberculin reactivity occurs as a result of remission by X-irradiation, alkylating agents or steriod therapy (27).

Cellular transfer of Hypersensitivity

With Lawrence's (17) demonstration of transfer of tuberculin sensitivity by use of immunocompetant cells of humans sensitive to tuberculin, it was not long before workers began to utilize this finding attempting to demonstrate a response to cellular transfer of hypersensitivity in sarcoidosis and Hodgkin's (12). The technique employed as in Lawrence's original work was by intradermal injection of dense suspensions of peripheral white cells taken from tuberculin-positive donors and testing the individuals in the same sites one day later with tuberculin in Sarcoid patients. Cellular transfer in Hodgkin's patients consisted of cells from donors who were sensitive to various test materials of microbial origin and in a few instances to DNCB. Almost universal success was encountered in sensitizing normal recipients as was that to patients with Sarcoid, at least on a transient basis, with reversion to anergic state at 3 to 4 weeks later (29). In Hodgkin's disease, in contrast, attempts at adoptive transfer

by living cells have so far failed.

## Transfer Factor

Lawrence's use of disrupted cells gave results equivalent to those obtained by the use of intact lymphocytes in transfer of delayed hypersensitivity. The active principle was termed "transfer factor". Transfer factor can be freed from "competent" lymphocytes from the peripheral circulation by (a) water lysis or (b) soaking the cells in specific allergen, thereby serving essentially complete removal without disruption of cellular integrity. "Transfer factor" has been found to be a dialyzable, non-protein substance with a molecular weight of 10,000 which is not alterable by DNase or RNase (29).

Baram and Masko (6) have reported a dialysable substance in peripheral WBCs from PPD sensitive human donors that is capable of causing the conversion of PPD negative individuals to a positive state. This appears also to be "transfer factor" as reported by Lawrence, but allows some con jecture as to a possible antibody molecule sub-unit of the cell.

## Degrading Immunocompetant cells

It would appear that this very significant finding in Hodgkin's patients of failure of cellular transfer of hypersensitivity by immunocompetent cells may well be a corrélary to the failure of the patient's own once immunocompetent cells. Therefore it is imperative to summarize Chase's (7) ideas on degradation of

immunocompetent cells. He reasons that with failure of cellular transfer of hypersensitivity in the patient with Hodgkin's disease, some mechanism must exist whereby the immunocompetent cells or transfer factor is rendered inactive. He ventures 4 possible mechanisms and makes his case for a possible plasma factor other than antibodies which degrades competent cells. He rejects the possibility of complete immunocompetence of the cells in Hodgkin's disease on the basis of available mechanisms of cell destruction. These are (a) contactual agglutination - attachment of patient's lymphocytes or macrophages to the donated cells serving as the target cell and (b) an interaction that results in injury and eventual cytolysis of both the target cell and the attacking cell. This has been noted to occur by the use of lymphocytes from specifically sensitized animals and by use of peritoneal macrophages not lymphocytes and "is difficult to conceive that these mechanisms could be used by the Hodgkin's patient with the high degree of anergy with respect to delayed type sensitivities". He also doubts the possibility of continuing synthesis of an antibody directed against all white cells since autoantibody has been detected in Hodgkin's patients only with associated hemolytic anemia. Likewise the concept of immunologic unresponsiveness or "paralysis" is rejected.

Non-antibody plasma factor

A good case is made for possible presence of a non-antibody plasma factor that acts to degrade immunocompetent cells and perhaps affect "transfer factor" as well. Experiments employing immunocompetent cells incubated with mitomycin C (which blocks production of new messenger RNA) showed that after stored RNA and DNA were used, hardly any sensitivity was exhibited to allergen in injected animals. Similarly, incubation of immunocompetent cells with ribonuclease, which also interfers with protein synthesis, but does not cause cell death, resulted in a pronounced decrease in capacity to elicit hypersensitivity reactions. This offers evidence that competent cells can be altered by special environmental factors and yet remain alive. Chase thus establishes a basis for a possible plasma factor that degrades immunocompetent cells. This seemingly opens the door for many avenues of continued and extended research in this area and has possibilities of discovery even beyond that of adding to an enlightenment of anergy in Hodgkin's.

The status of the lymphocyte-cellular responses

The cultivation of immunocompetent lymphocytes with antigen has been accomplished and results interpreted as representing delayed hypersensitivity. In one report (5) lymphocytes from tuberculin-sensitive persons, cultured with tuberculin IN VITRO were found

to be stimulated to increase in size and divide. Response was measured in terms of the number of blast cells plus cells showing mitotic figures formed in 5 days. Various antigens employed include tuberculin (PPD), pertussis vaccine, tetanus toxoid, diphtheria toxoid, and penicillin. Addition of crude phytohemagglutinin causes even more dramatic responses. It has been debated whether this represents a pure delayed type hypersensitivity however. Assuming that this represents delayed sensitivity, studies by Hirschhorn (14) have shown that in patients with Hodgkin's and Sarcoidosis there is a "reduced or absent responses of their lymphocytes to specific antigens IN VITRO, mimicking their anergy IN VIVO".

Hirsh and Oppenheim (13) demonstrated that PHA was much less effective as a mitotic agent for inducing blast forms in the cells of patients with Hodgkin's than it is for normal cells, and that anergic patients with Hodgkin's disease showed less transformation with PHA than those who responded to at least 1 intradermal test material. This demonstrates that PHA response was clearly associated with the cell. However, on examination of plasma factors, a suspension of normal cells was added to the plasmas of 3 patients whose cells-plus-plasma showed impairment of PHA stimulation. In two cultures of this cell sample, mitogenic responses were normal. In the 3rd culture, the cells died in the presence of the patients plasma, a finding that led to a further

experiment of interesting outcome. The cells of the patient furnishing this plasma were transfered to normal plasma and were here found to acquire partial responsiveness to PHA. This suggests that plasma factors may be additive at times in determining a low PHA response, and adds some basis for Chase's concept of a plasma factor.

The Thymus

The Thymus has been shown to control the distribution of lymphocytes in the very young and has been thought to exert a measure of control even in the mature. It has been suggested to have a role in sustaining the mechanism of immunologic unresponsiveness. Thymectomized neonatal animals exhibit anergy among other immunologic abnormalities and thus warranted attention on the basis of a possible similar existing mechanism in Hodgkin's. There are some corresponding conditions in the thymectomized animal to the wasting condition which is characterized by lymphocytopenia, severe depletion of tissue lymphocytes, diarrhea and eventual death (11). Recent evidence however, particularly the absence of wasting in thymectomized germ-free animals, points to an infectious etiology of this syndrome (19). Also it seems unlikely that the thymectomy-wasting syndrome could account for the form of presentation of localized Hodgkin's disease, the histologic picture (Reed-Sternberg cells) or the chills, fever and leukocytosis of the disseminated disease (4). Aisenberg (4)

further found that thymectomy beyond the neonatal period does not lead to significant immunological impairment. Other observations in the neonatally thymectomized animal in contrast to the Hodgkin's patient are those of frequent occurrence of early lymphocyte depletion and a depression of antibody formation which may parallel the depression of delayed hypersensitivity.

Homograft reaction

There is not general agreement among authorities about the homograft reaction and it's possible corrolary to anergy. Rejection of skin homografts is mediated by a cellular mechanism under most conditions and in Hodgkin's patients, skin homografts survival rates are reported to be abnormal in that over one half survive 30 days or longer (15). Similar findings in disease states that involve antibody depression clouds any clear cut conclusions. It is interesting that in animal studies, lymphoid cells incubated IN VITRO with homologous RNA has resulted in a heightened immunity to skin homografts (20). This may implicate a deficiency of sorts of RNA at the cellular level in homograft rejections in Hodgkin's disease.

Another line of study involving tissue transplant is that of observation of cellular response occurring in draining lymph nodes. The general reaction is that of large lymphoid cell formation occurring in the cortex 3 to 4 days later by virtue of their transformation from small lymphocytes. Particular interest has

developed in that animals rendered immunologically unresponsive to sensitization with DNCB have failed to develop any significant increase of the large lymphoid cells. This may represent a prototype of anergy, and further studies may be rewarding (7).

## Summary

Delayed hypersensitivity has been reviewed. The single most important concept is that of antigen distribution and the postulation of its induction role on the lymphocyte from its depot site in lymphoid follicles.

The basic defect causing anergy in Hodgkin's disease appears to be that of abnormal lymphocyte function as evidenced by abnormalities exhibited by Hodgkin's lymphocytes in cell culture and transfer studies.

Anergy in Hodgkin's patients is somewhat unique in that it occurs early in the disease state as opposed to "cachetic anergy" in other neoplastic diseases. As compared to anergy in Sarcoidosis, there is no reversibility of the anergic state on a temporary basis indicating that anergy seems to be more profound in Hodgkin's disease.

Induction of anergy after disease onset has been observed to vary from 2 to 8 months. Reconversion to delayed reactivity occurs as a result of remission by X-irradiation, alkylating agents and steroid therapy. Conversions to positive reactions occures uniformly in patients where the disease is inactive for

a period greater than two years, and inconsistently where the disease is inactive from 2 to 24 months.

Attempts of cellular transfer of immunocompetent cells and "transfer factors" in Hodgkin's patients have failed. This seems to imply the existance of some mechanism that degrades immunocompetent cells and or "transfer factor". There is some evidence that possibly a non-antibody plasma factor may be responsible for the degradation of the immunocompetent transfer cells and may well represent the cause of failure of the patient's own once immunocompetent cells.

The study of thymectomized neonatal animals and homograft reactions in animals seems to substantiate the role of the lymphocyte in anergy. There are indications of cellular RNA deficiencies in Hodgkin's lymphocytes in homograft studies.

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