

The Indirect Basophil Test in Erythema Nodosum Leprosum

Preliminary Note^{1,2}

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Erythema nodosum leprosum (ENL) has been described as an allergic reaction to organisms *in situ* or to drugs or toxins carried by the bloodstream. If this reactional phenomenon continues, the patient can develop panniculitis nodosa and finally the most serious state, the progressive reaction. Although erythema nodosum leprosum can occur "spontaneously," there is no doubt of the greater incidence since the advent of the more effective chemotherapeutic agents, e.g., sulfones, thiosemicarbazones, thioureas, and long-acting sulfonamides.

Gokhale and Joglekar (²) reported that they could differentiate the lepra reactions caused by the sulfones from those that occur spontaneously by using the indirect basophil degranulation test. In an attempt to duplicate their results and possibly to explore other potential immunologic phenomena in leprosy with the indirect basophil degranulation test, blood serum was drawn from five patients with erythema nodosum and sent from the U. S. Public Health Service Hospital, Carville, Louisiana, to the U. S. Naval Hospital, Philadelphia, Pennsylvania, by air express. Three of the specimens were from patients whose reaction was associated with dapsone (DDS) therapy; the other two were from patients whose reaction occurred "spontaneously."

METHODS AND RESULTS

The specimens were labeled so that the technicians were unaware of the source of

the material. The technic used was the indirect three drops supravital slide technic of Shelley (⁷) with the minor modification of Katz *et al.* (⁴).

Dapsone is a white, odorless, crystalline powder which is practically insoluble in water. Gokhale and Joglekar (²) used ether as the solvent for their antigen dapsone (1:1,000 dilution). In our indirect basophil degranulation tests, when their antigen preparation was added, it degranulated the rabbit's basophils not only in the serum of each leprosy patient but also in the serum of each control. It was then demonstrated that when the buffy coat of each rabbit's blood was mixed with a drop of ether, there was significant degranulation of the basophils. The insoluble dapsone powder was made a little more soluble in water and saline by heating; however, these suspensions and unheated ones were incapable of causing any significant degranulation of the basophils in the test or control preparations. Using only crystalline dapsone powder as the antigen, we noted no significant results at the test and control sites on the slides.

After consulting with Shelley (⁸), we fed the rabbits daily doses of 400 mgm. of dapsone for two weeks. By utilizing the method of Bratton and Marshall (¹), we were able to demonstrate significant blood levels of dapsone in the rabbits, more than 1 mgm./100 ml. On examination of the buffy coat of the blood of these rabbits, no significant degranulation or alteration of basophils was seen. When the "dapsone-coated" basophils were added to the serum of each patient, no significant morphologic changes were noted in these cells.

Throughout these studies, each patient's serum was tested four times with each antigen.

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² The views expressed herein are those of the authors and do not necessarily reflect the view of the United States Navy, or the Department of Defense, or the United States Public Health Service.

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DISCUSSION

In this brief and preliminary study, the important findings were: (1) that the degranulation of basophils observed by Gokhale and Joglekar⁽²⁾ using dapsone dissolved in ether as antigen was reproducible when ether alone was used; and (2) that the degranulation of basophils with the ether-dissolved antigen occurred in the serum of the patients and the controls.

In the immunologic spectrum of leprosy, there is a concept that erythema nodosum leprosum may be due to circulating anaphylactoid antibodies. When patients with leprosy receive dapsone (DDS) and experience a lepra reaction, the dapsone could behave as a haptene that couples with protein and stimulates the formation of circulating antibodies of the immediate type. Since mast cells are fairly numerous in lepromatous tissue⁽³⁾, it has been suggested that an antigen-antibody complex may result in the degranulation or disintegration of these mast cells with the liberation of histamine, which incites the changes of an allergic type of inflammation which we appreciate clinically as erythema nodosum leprosum⁽⁵⁾.

If the patients receiving dapsone had developed circulating antibodies because of this therapy, the failure to obtain a positive indirect basophil degranulation test in these patients might have been due to the following reasons: (1) Dapsone therapy resulted in the formation of a metabolite, breakdown product, or haptene protein-complex which is the true and untested antigen. (2) The level of circulating antibodies in the tested sera was below that requisite for detection by the basophil test. This explanation has been offered for the relatively unsatisfactory clinical correlation of allergic drug eruptions of the erythema multiforme, morbilliform, and nodosum types with the indirect basophil degranulation test⁽⁹⁾. (3) In spite of our efforts to reduce to a minimum the time from the drawing of the blood to the performance of the tests and the potential adverse temperature factors, the sera were not processed within the one hour time limit which Shelley⁽⁹⁾ regards as essential for a valid

result. (4) The insolubility of dapsone in water or saline could have resulted in too weak an antigen. (5) The sample size, two and three sera respectively, was too small for significant results.

Perhaps the erythema nodosum leprosum is due to a delayed type of hypersensitivity. In guinea-pigs, Raffel and Forney⁽⁶⁾ noted that they could induce delayed types of reaction to drugs such as picryl chloride provided that they were combined with purified wax fractions of the tubercle bacillus. Can one infer that in patients with lepromatous leprosy the lipid fraction of the leprosy bacillus can alter the reactivity of the host toward dapsone so that the delayed type of allergic reaction of the erythema nodosum type occurs?

SUMMARY

In this brief and preliminary study, the authors were unable to duplicate the work of Gokhale and Joglekar, who reported that lepra reactions caused by dapsone could be differentiated from those that occur spontaneously by using the indirect basophil degranulation test. Because of the reasons presented in the discussion, the authors are unable to speculate on the presence or absence of circulating antibodies to dapsone in this study.

RESUMEN

En este estudio breve y preliminar, los autores no pudieron reduplicar el trabajo de Gokhale y Joglekar, quienes comunicaron que las reacciones de la lepra causadas por la dapsona, pudieran ser diferenciadas de aquellas que ocurren espontáneamente usando la prueba de la degranulación basofila indirecta. Por causa de las razones presentadas en la discusión los autores no pudieron especular, en este estudio sobre la presencia o ausencia de anticuerpos circulantes a la dapsona.

RÉSUMÉ

Au cours de cette courte étude préliminaire, les auteurs n'ont pas réussi à reproduire le travail de Gokhale et Joglekar, qui ont signalé que les réactions lépreuses produites par la dapsone pouvaient être distinguées de celles survenant spontanément, au moyen d'une épreuve de dégranulation basophile indirecte. A

la suite des arguments présentés dans cette discussion, les auteurs ne sont pas en mesure d'émettre dans ce travail des spéculations quant à la présence ou à l'absence d'anticorps circulants contre la dapsoné.

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