to that found with cyclophosphamide (Fox, 1964) and better than that found with Amethopterin (methotrexate) (Medawar, 1963) or mercaptopurine (Meeker *et al.*, 1960).

The mechanism by which thalidomide produces prolongation of skin-graft survival is not clear. It may be through a direct immunosuppressant action on the lymphoid system, though this is rendered less likely by the finding (see Table) that treatment of the donor before transplantation as well as the recipient increases length of graft survival. It seems more probable that, if a specific effect on the immune system is involved, thalidomide modifies the donor skin antigen or its recognition.

The findings of Roath *et al.* (1962, 1963) that thalidomide inhibited the phytohaemagglutinin-induced transformation of human lymphocytes into "blast" cells might also indicate that thalidomide could be expected to have an immunosuppressive action. Some doubts about these findings were raised by Lindahl-Kiessling and Böök (1963), though these authors gave insufficient details of their experiments to allow assessment of the validity of their criticism. In the light of the present findings this problem might usefully be looked at again.

These experiments of Roath *et al.* also led Playfair *et al.* (1963) to test oral thalidomide on skin homograft survival in mice; they, however, failed to observe any significant increase. It is difficult to know to what to attribute the disparity between their results and those reported here, since these authors too give virtually no experimental detail. It is unlikely to be due to the different route of administration which they employed, since in one experiment (not included in the Table because of excess mortality unrelated to the experiment) increased graft survival after oral thalidomide was also noticed in the two surviving mice of this experiment.

The timing of the thalidomide doses is of some importance. The best results in the present experiments were obtained when the recipients (as well as the donors) were pretreated. In this respect thalidomide is comparable to radiation, radiomimetic drugs, and cortisone-like steroids, which are most effective as immunosuppressants when given before an antigenic stimulus (Hitchings and Elion, 1963).

Timing was also found to be important by Tata (1964), who showed that thalidomide is toxic to the tadpole for a short period in metamorphosis. It therefore seems likely that thalidomide might act only at a particular time in cell division. Because it is sparingly soluble in aqueous media and because of its instability the problem would be to have a sufficient concentration of the compound available to sensitive cells.

Although more information has been obtained with the $C_{57}BL—C_3H/He$ combination than with $A—C_3H/He$, it appears that the latter combination gives better prolongation than the former. This again might be expected if thalidomide were acting as an immunosuppressant.

Thus in a limited way the present experiments provide support for the idea that thalidomide might possess immunosuppressive properties.

Conclusion

Thalidomide prolongs skin homografts in mice at non-toxic doses. The best results were obtained when the donor graft was treated with thalidomide either *in vivo* or *in vitro* in addition to the recipient.

These findings are discussed and lend some support to the idea that thalidomide may have an immunosuppressive action.

We are much indebted to Professor L. Brent for showing us the transplantation technique he uses and to Sir Peter Medawar for a useful discussion. We would like to thank Mr. Ade Olukoya and Mr. Peter Hodgson for their technical help. We are particularly grateful to Dr. M. Perelman and Dr. S. S. Szinai, of Lilly Research Laboratories Ltd., for generous supplies of thalidomide, and also to Dr. G. F. Somers, of Reckitt & Sons Ltd.

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L.E. Cells and Antinuclear Factors in Leprosy*

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Brit. med. J., 1965, **2**, 689–690

The suggestion has been made of the possible involvement of autoimmune mechanisms in some aspects of leprosy—namely, lepra reactions (Chini, 1961). A number of autoimmune-like reactions have been reported to occur in leprosy (Cathcart *et al.*, 1961; Rizzi *et al.*, 1962; Bonomo *et al.*, 1963). We were therefore particularly interested in investigating sera from leprosy patients for the presence of lupus erythematosus (L.E.) cells and antinuclear factors (A.N.F.) by means of the fluorescent-antibody technique.

Material and Methods

The sera of 55 unselected patients with leprosy (lepromatous type) in hospital at the Hansenian Colony, Gioia del Colle, Bari, Italy, were tested for A.N.F. Blood specimens from 10 leprosy patients whose sera were found to contain A.N.F. were examined for L.E. cells as well.

Fluorescent Test for A.N.F.—The indirect technique described by Weir *et al.* (1961) was employed, with slight modifications (Bonomo *et al.*, 1965). Sera were tested in serial dilutions with buffered saline (*p*H 7.2), and those which showed fluorescent reactions in dilutions beginning from 1:2 up were

* This investigation was supported in part by Consiglio Nazionale delle Ricerche (Immunopatologia), Roma.

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considered to be positive. With this method the incidence of a definite positive reaction in sera from normal individuals is 1%.

L.E. Cell Test.—For this test the direct method as described by Zinkham and Conley (1956) was used.

Results

The Table shows the results of A.N.F. and L.E. tests (first testing) of specimens of blood from the leprosy patients. The tests were not repeated. In the three definitely positive cases the L.E. cell preparations contained numerous typical L.E. cells. "Rosette" formation and nuclear inclusions were found in the fourth case.

Incidence of A.N.F. and L.E. Cells in Leprosy

A.N.F.			L.E. Cells		
No. Tested Sera	Positive		No. Tested Blood Specimens	Positive	
	No.	%		No.	%
55	16	29·09	10	3 (typical)	30 } 40
				1 (rosette)	10 }

It seems likely that systemic lupus erythematosus was associated with leprosy in one of the patients who had L.E. cells and A.N.F. None of the others with A.N.F. or L.E. cells had another disease which could have been responsible for such reactions.

The occurrence of A.N.F. or L.E. cells had no relation to the age distribution of the patients or to the duration of the disease. Three of the patients with L.E. cells were females and one was a male; nine of those with A.N.F. were males and seven were females.

Discussion

The finding of L.E. cells and A.N.F. provides additional evidence that immunological reactions occur in leprosy. Indeed, several of the "markers" of an autoimmune disease (Mackay and Burnet, 1963) are known to occur—namely, autoimmune reactions, hypergammaglobulinaemia, cell-bound hypersensitivity to *Mycobacterium leprae* constituents, and systemic reticuloendothelial involvement.

The simultaneous occurrence of different autoimmune reactions was observed in 11 (69%) of the 16 patients with A.N.F. Indeed, rheumatoid factor, thyroglobulin antibodies, and "false" positives in the serological tests for syphilis occurred respectively in 11, 7, and 6 out of the 16 patients with A.N.F.

This simultaneous occurrence of different autoimmune activities (Bonomo *et al.*, 1963) gives further support to the belief that exaggerated sensitivity of the antibody-forming system may be responsible for development of the autoimmune pattern observed in leprosy.

The sustained antigenic stimulation due to chronic infection by *M. leprae* may represent the mechanism responsible for the autoimmune activity in leprosy. Indeed, antinuclear factors have recently been described in rabbits hyperimmunized with killed bacteria (Christian *et al.*, 1963). Likewise, the effect of a prolonged antigenic stimulus seems relevant to the production of rheumatoid or rheumatoid-like factors (Abruzzo and Christian, 1961; Williams and Kunkel, 1962). In particular, an adjuvant-like effect of the chronic infection due to *M. leprae* may to a large extent contribute to the establishment of hypersensitivity and the development of autoimmune cellular mechanisms.

A better understanding of the factors responsible for the autoimmune reactions observed in leprosy and other diseases with known aetiology may help to elucidate the aetiological mechanisms of more obscure diseases. In this light, diseases of known aetiology with autoimmune factors may be considered to be true experiments of nature.

On the other hand, at this stage of investigation and available data it is not possible to decide whether the establishment of the autoimmune mechanism here discussed is an epiphenomenon, secondary to the pathological processes of the disease, or whether it has some pathogenetic bearing on one or other of the protean aspects of leprosy.

Summary

Serum antinuclear factors as detected by the immunofluorescence method were found in 16 out of 55 cases of leprosy.

L.E. cells or "rosettes" were present in the blood specimens of 4 out of 10 leprosy patients with serum antinuclear factors.

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