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Prolonged Survival of Skin Allografts in Leprosy Patients[1,2]

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It is generally recognized that immunologic abnormalities occur in leprosy patients, especially those with the lepromatous form of the disease. Excellent discussions of these abnormalities are included in the recent reports of Waldorf and associates (14), Dierks and Shepard (6), Bullock (2), Sheagren and associates (12), and Turk and Waters (13).

In lepromatous leprosy the "nonspecific immunologic abnormalities" reported include a moderate to markedly lowered capacity to develop delayed sensitivity to antigens unrelated to *Mycobacterium leprae* [4] a depressed lymphocyte transformation response to phytohemagglutinin (PHA) and antigens such as tuberculin and streptolysin O; high serum levels of the immunoglobulins IgG and IgA; elevated clearance capacity of the reticuloendothelial system; and a marked depletion of thymus-dependent lymphocytes in the paracortical areas of the lymph nodes.

In tuberculoid leprosy nonspecific immunologic abnormalities are less marked than in lepromatous leprosy.

There is no convincing evidence that antimicrobial cellular immunity is nonspecifically depressed in leprosy, for leprosy patients are not known to be especially susceptible to other diseases such as tuberculosis.

In view of the observation that antitissue cellular immunity and delayed sensitivity appear to be obligatorily interdependent and that the rejection of primary allografts results largely from cellular immune forces, it is reasonable to expect that patients with leprosy would exhibit an impaired immune response to allografts.

To date there have been few attempts to measure the immune response to allografts in leprosy. To our knowledge the only journal report is that of Mercau and Albertengo, quoted by Rodriguez Paradisi and

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4 The term delayed sensitivity is used in preference to the more commonly used term "delayed hypersensitivity."

associates ([11]), which was not available to us, in which it was stated that lepromatous patients tolerate homografts better than normal individuals. Hart and Rees ([8]) have referred to unpublished work by Job and Karat in which four lepromatous patients received skin grafts from other lepromatous patients. Three of the grafts survived for 19, 20 and 30 days respectively. The fourth graft was reported to be still viable at 70 days; however, it is possible that autologous epithelialization may have occurred and have been mistaken for a surviving graft, a not uncommon error. Presumably normal skin was not grafted to lepromatous patients nor were comparative control studies done using tuberculoid patients or normal subjects as graft recipients.

The present investigations were conducted to explore immunologic impairment in leprosy with respect to the capacity of patients with the two polar forms of leprosy, lepromatous leprosy and tuberculoid leprosy, to reject skin allografts from normal donors.

MATERIALS AND METHODS

The study was conducted using 30 young adult male volunteers; 10 were patients with lepromatous leprosy; 10 were patients with tuberculoid leprosy; and the remaining 10 were normal healthy adults. All patients were on DDS treatment that had been initiated at various intervals ranging from months to years before the experiment was conducted. All subjects were well-nourished and their general physical condition was good. It is improbable that differences in general nutrition, or DDS therapy influenced the survival of grafts in the various groups. The subjects were divided into groups of six and grafting was performed on five different occasions, each of which involved the members of one group (Fig. 1). Grafting within each group consisted of reciprocal skin grafts transferred between the two normal subjects and two skin grafts transferred from each of the two normal subjects to patients, one to a lepromatous patient and the other to a tuberculoid patient. All donors were carefully screened for a history indicative of viral hepatitis and no donor with a serum glutamic-oxaloacetic-transaminase (SGOT) titer exceeding 40 units was included in the study. Major blood grouping was conducted but histocompatibility testing was not done. The experimental design is illustrated diagramatically in Figure 1.

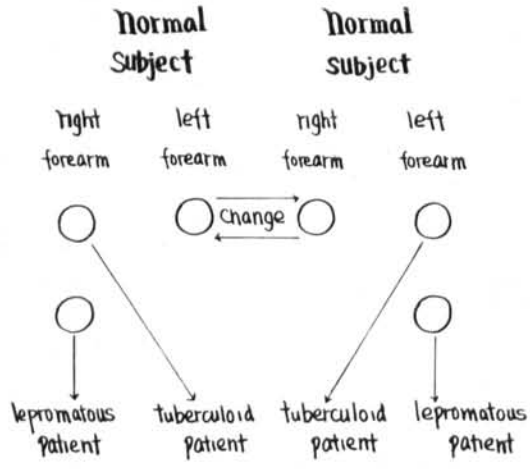

FIG. 1. Design of the skin allograft experiments.

The method of skin grafting employed was essentially the same as the method described by Converse and Rappaport ([4]). The grafts were removed from and applied to the mid-volar surface of the forearm. The skin surrounding the graft sites was healthy in appearance. Under local anesthesia, the donor site was incised with a circular trephine (11 mm. in diameter) and the graft was excised from underlying tissue. Each graft was placed on gauze saturated with physiologic saline, and the undersurface was freed of subcutaneous tissue. After bleeding and capillary oozing from the recipient graft bed had ceased, the edges of the graft were approximated to the edges of the graft bed with interrupted 5-0 nylon sutures. The grafts were then covered with a layer of fine nylon mesh, and pressure dressings were applied. Unused graft beds were closed with sutures. The grafts were observed daily and the sutures were removed on the seventh day.

The status of the grafts was evaluated on the basis of gross findings, and, in a few randomly selected cases by histologic examination of biopsied specimens taken early during graft rejection. Grafts were defined as "rejected" when two-thirds or

more of the graft showed evidence of circulatory stasis as indicated by intense cyanosis. Within two days most of such grafts developed a brownish eschar covering regenerating epithelium.

Bacterial indices were determined from skin smears from all leprosy patients ([10]).

RESULTS

On the fifth postoperative day, 28 of 30 grafts had "healed in" and were pink. The two graft failures, one a graft on a tuberculoid patient and the other a graft on a normal control subject, were apparently due to lack of cooperation on the part of the recipients; the results are not included in this report.

In normal recipients, the pink color of the graft deepened after the sixth day to become dark red and finally cyanotic. By the 11th day, two-thirds or more of the graft area of all but two of the grafts on normal recipients were deeply cyanotic in appearance, i.e., had been rejected (Table 1) On the 11th day the grafts on all but two of the leprosy patients were still fully viable.

All but two of the grafts on tuberculoid patients were rejected by the 14th day. In marked contrast to the grafts on normal subjects and tuberculoid patients, only two of the grafts on patients with lepromatous leprosy were rejected by the 14th day.

Early after the first signs of impending rejection a representative graft from a recipient of each group was biopsied for histopathologic study. Rejection was always accompanied by round cell infiltration, thrombosis, hemorrhage, and separation of the graft from its bed. The histopathologic appearance was essentially the same in normal subjects and in patients with the two types of leprosy.

The mean survival time of the grafts on normal recipients was 11.22 days (range 9-15 days, median 11 days) with a standard deviation of 1.99. Allograft rejection was delayed appreciably in patients with lepromatous leprosy, the mean survival time of grafts being 15.20 days (range 12-18 days, median 15 days) with a standard deviation of 1.87. Prolonged survival of grafts was less marked in tuberculoid patients, the mean survival time being 13.44 days (range 11-16 days, median 14 days) with a standard deviation of 1.66. Student's t-test analysis showed that the differences in mean survival times were significant for all groups, i.e., between grafts on lepromatous patients and normal subjects; tuberculoid patients and normal subjects; and lepromatous patients and tuberculoid patients.

The results of bacterial indices determined from leprous skin lesions (distinct from graft sites) showed that graft survival

TABLE 1. *Survival times of skin allografts on leprosy patients and normal subjects.*[a]

Recipient No.	S.T.[b] days	Recipient No.	S.T.[b] days	Bact.[c] index	Recipient No.	S.T.[b] days	Bact.[c] index
N1	10	T1	14	0	L1	15	2.3
N2	10	T2	14	0	L2	15	4.7
N3	11	T3	13	0	L3	14	0
N4	10	T4	11	0	L4	13	2.7
N5	11	T5	11	0	L5	12	0
N6	14	T6	(failure)		L6	17	5.0
N7	11	T7	16	1.2	L7	15	3.6
N8	9	T8	14	0	L8	15	4.0
N9	15	T9	15	2.4	L9	18	3.6
N10	(failure)	T10	13	0	L10	17	3.8
Mean 11.22 ± 1.99 days		Mean 13.44 ± 1.66 days			Mean 15.2 ± 1.87 days		

[a] N = normal subject; L = lepromatous patient; T = tuberculoid patient.
[b] S.T. = survival time.
[c] Bact = bacterial.

was directly related to the presence of organisms in the lesions; with one exception (L4) all patients who showed bacilli in skin lesions bore grafts that survived 15 days or longer and in no instance in which the graft survived 15 days or longer were organisms absent.

DISCUSSION

The present observation that allograft immunity is impaired in both the lepromatous and tuberculoid forms of leprosy, albeit to different degrees, is in conformity with expectation based on existing knowledge relative to the role of lymphocytes in delayed sensitivity and antitissue cellular immunity, and known abnormalities of the lymphocytes of leprosy patients ([13]). A normal responding lymphocyte appears to be required for the development and expression of delayed sensitivity and antitissue cellular immunity; deficiencies or failure of these responses are commonly, if not invariably, accompanied by deficiencies in lymphocyte function measurable in different ways.

Lymphocyte deficiencies which have been detected in leprosy include: a depressed lymphocyte blastogenic response to PHA and antigens of common human pathogens ([3, 6]), and, as we have found a depressed capacity to produce lymphotoxin when stimulated with PHA or leprolin ([7]) and a decreased capacity to cause inhibition of migration of macrophages when stimulated by specific antigen ([7]).

Delayed sensitivity reactions depend in large measure on the activities of lymphocytes. In lepromatous leprosy, Wayson ([15]) was among the first to call attention to a low cutaneous sensitivity to tuberculin; Buck and Hasenclever ([1]) found a low cutaneous sensitivity to *Candida albicans*; Waldorf and associates ([14]) failed to induce delayed sensitivity to dinitrochlorobenzene in 50 per cent of cases of lepromatous leprosy; and Bullock ([2]) observed a low capacity to develop delayed sensitivity to picryl chloride.

The relations which these manifest lymphocyte deficiencies may bear to resistance to leprosy are not known.

A notable observation is that specific immunologic deficiencies in lepromatous leprosy are more profound than nonspecific immunologic deficiencies; for example, delayed sensitivity reactions to lepromin are very weak or absent and specific cellular immunity to *M. leprae* is evidently severely depressed, for growth of the organisms within macrophages appears to occur with little or no restraint.

Specific cellular immune responses are regarded to be greater in tuberculoid leprosy than in lepromatous leprosy (as evidenced by positive skin reactivity to lepromin and greater resistance to growth of the organisms) and have been generally assumed not to be subnormal in tuberculoid leprosy. However, this cannot be defended with certainty and it is possible that the tuberculoid patient as well as the lepromatous patient is subnormal in his specific cell-mediated immunologic responses to *M. leprae* as suggested by the fact that he has developed the disease, whereas other equally exposed have escaped. Indeed it may be that the tuberculoid patient is both specifically and nonspecifically subnormal in his immunologic responses, albeit to a lesser degree than the lepromatous patient.

Whereas the moderate to marked nonspecific immunologic impairment seen in lepromatous leprosy could be due to the infection itself (which may evoke no surprise because the enormous numbers of organisms in the tissues might be expected to somehow overwhelm the immune apparatus) it would be surprising if significant nonspecific immunologic impairment were to occur in tuberculoid leprosy as the result of direct effects of the very small numbers of organisms on the immune apparatus.

These reflections suggest the possibility that all individuals who develop leprosy are inherently subnormal to one degree or another, not only with respect to their specific immunologic responses to *M. leprae* but to antigens in general; especially responses related to granuloma formation, delayed sensitivity and cellular immunity. The concept that susceptibility to leprosy rests on a lowered capacity to respond specifically to *M. leprae* is supported by a most remarkable observation, namely, that the Mitsuda-negative patient with diffuse lepromatous leprosy seldom if ever de-

velops a persisting state of true Mitsuda type positivity to lepromin at any time, even after arrest or cure of the disease ([9]), and by the additional observation of Dharmendra and Chatterjee ([5]) that normal individuals who are persistently negative to repeated lepromin tests and who contract leprosy appear to always develop the lepromatous form of the disease.

The extent to which both specific and nonspecific immunologic abnormalities in leprosy may result from genetically-determined immunologic defects existing before infection on the one hand and to the effects of the infection itself on the other hand is not readily apparent or easily determined. This important problem is receiving increasing attention and there is a growing conviction that genetically-determined subnormal immunologic responsiveness characterizes both tuberculoid and lepromatous leprosy ([2]). For example, with respect to mechanism, lack of specific delayed sensitivity and specific antimicrobial cellular immunity in lepromatous leprosy could result from a specific genetic defect governing macrophage function or the capacity of lymphocytes to respond to an important immunogen of *M. leprae* and secondarily could involve acquired immunologic tolerance, immunologic enhancement, immune deviation, or desensitization. On the other hand nonspecific abnormalities involving cellular immunity and delayed sensitivity could be due to competition of antigens, or cytotoxic or other effects produced by the organisms. Both specific and nonspecific depression of cell-mediated immune responses could result, in part at least, from depletion of thymus-dependent lymphocytes.

SUMMARY

The comparative survival times of allografts of normal skin were studied in 30 well-nourished young, male volunteers, including normal subjects and patients with tuberculoid and lepromatous leprosy.

Allograft immunity was impaired significantly in the recipients with tuberculoid and lepromatous leprosy, impairment being greatest in lepromatous leprosy. The possible significance of these results with respect to immunologic events in leprosy and the altered immunologic potential of leprosy patients is discussed.

RESUMEN

Se estudiaron comparativamente los tiempos de sobrevida de aloinjertos de piel normal en 30 voluntarios varones, bien alimentados, incluyendo sujetos normales y pacientes con lepra lepromatosa y lepra tuberculoide.

La inmunidad contra los aloinjertos estaba significativamente deteriorada en los receptores con lepra tubercu oide y lepromatosa, siendo el deterioro más acentuado en lepra lepromatosa. Se discute el posible significado de estos resultados con respecto a los hechos inmunológicos en la lepra y el potencial inmunológico alterado de los pacientes con lepra.

RÉSUMÉ

Chez 30 jeunes volontaires de sexe masculin, bien nourris, parmi lesquels on comptait des individus normaux et des malades souffrant de lèpre tuberculoide et de lèpre lépromateusei on a procédé à une étude comparative des temps de survie d'allogreffes de peau normale.

L'imminuté aux allogreffes était significativement altérée chez les sujets présentant une lèpre tuberculoide ou une lèpre lépromateuse. C'était chez ceux qui étaient infectés de lèpre lépromateuse que cette altération était la plus prononcée. On discute de la signification possible de ces résultats par rapport aux phénomènes immunologiques qui surviennent dans la lèpre, et ceci dans la perspective d'une altération des capacités immunologiques chez les lépreux.

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