INTERNATIONAL JOURNAL OF LEPROSY

Volume 48, Number 2
Printed in the U.S.A.

Variations in Dinitrochlorobenzene Responsivity in Untreated Leprosy: Evidence of a Beneficial Role for Anergy[1]

Thomas H. Rea and Norman E. Levan[2]

Anergy in leprosy—a generalized impairment of cell-mediated immune (CMI) responsivity toward antigens other than those of *Mycobacterium leprae*—has been widely recognized. This anergy has been perceived by several measures of CMI responsivity: by direct means, specifically elicitation of tuberculin-type delayed hypersensitivity ([3,26]), induction of allergic contact dermatitis ([3,26,31,33]) and rejection of allografts ([9]); by *in vitro* equivalents of CMI such as antigen-induced lymphocyte transformation ([10]); and by *in vitro* indices of CMI responsivity such as lectin-induced lymphocyte transformation ([5,10,16]) or peripheral blood T-lymphocyte enumeration ([6]) and classification ([15]). The anergic state has been more readily demonstrated in lepromatous than tuberculoid patients ([3,10,26,31]), and borderline patients appear to be intermediary ([26]).

However, the anergic state is not an invariable accompaniment of lepromatous leprosy ([3,31]). Furthermore, in some series only one or two of the several responses studied were impaired ([16,28,29,33]), and in other studies no evidence of anergy was found ([4,7,22,32]).

This irregular distribution of anergy, particularly among borderline and lepromatous patients, has been difficult to understand, proffered explanations having included the influence of treatment ([3]), associated erythema nodosum leprosum (ENL) ([33]), genetic factors ([16]), length of illness ([4]), endemicity ([7]), and fortuitous environmental factors ([11]) such as infection or malnutrition.

Although in our initial reports ([21,22]) we found no evidence of anergy (or an anergic subgroup) among lepromatous patients, dinitrochlorobenzene (DNCB) responsivity was perceptibly less (albeit insignificantly so) among untreated than in treated subjects ([21]). We therefore have continued to study DNCB responsivity, reasoning that this simple but powerful technique might identify an anergic subgroup among larger numbers of untreated patients and controls. We have found DNCB responsivity to be significantly impaired in untreated borderline and lepromatous patients, but in untreated patients with reactional states, this impairment was absent or greatly attenuated.

PATIENTS AND METHODS

Patients were classified as having tuberculoid, borderline, or lepromatous leprosy. Using the more precise criteria of Ridley ([24]), the tuberculoid patients were either polar tuberculoid or borderline with tuberculoid features; the borderline patients were either borderline or borderline with lepromatous features; and the lepromatous patients were either polar or subpolar lepromatous. With few exceptions, patients were managed entirely as outpatients. Testing was begun as soon as the diagnosis was established, prior to the institution of chemotherapy. Patients were accepted as untreated if repeated histories were negative, e.g., without suggestion of skin biopsy, skin scraping, use of a medication daily or every other day for prolonged periods, etc.

Three reactional states were recognized. "Reversal" reactions were identified by abrupt worsening of previously indolent lesions, usually accompanied by new skin lesions and the abrupt onset or worsening of

[1] Received for publication on 31 October 1979; accepted for publication on 4 January 1980.

[2] T. H. Rea, M.D., N. E. Levan, M.D., Section of Dermatology, Department of Medicine, University of Southern California and the Department of Dermatology, University of Southern California, Los Angeles County Medical Center, Los Angeles, California, U.S.A. Please send all correspondence to Dr. Rea, Section of Dermatology, USC School of Medicine, 2025 Zonal Ave., Los Angeles, California 90033, U.S.A.

nerve trunk palsies ([23]); without exception, cases diagnosed as reversal reactions required systemic corticosteroids for management. Because patients developing reversal reactions early in the course of chemotherapy are thought to actually have mild reversal reactions at the time of presentation ([1]), patients developing overt reversal reactions within the first 10 weeks of therapy were designated as having had incipient reversal reactions at the time of attempted DNCB sensitization. Erythema nodosum leprosum (ENL) was identified by crops of tender, red nodules arising in apparently normal skin, usually associated with fever and malaise, a neutrophilic infiltrate on histological examination, and a dramatic therapeutic response to thalidomide ([19]). Lucio's reaction was recognized by painful, non-tender, cutaneous, hemorrhagic infarcts occurring in diffuse non-nodular lepromatous disease and necrosis in association with endothelial proliferation on histological examination ([20]).

Excluded from this report were those patients presenting with reversal reactions warranting immediate corticosteroid therapy, borderline patients followed for less than 10 weeks, and those who were pregnant. None of the women patients were using oral contraceptives at the time of attempted DNCB sensitization.

Controls for DNCB responsivity were medical students, hospital personnel, and clinic patients judged to be in good health and on no medication known to influence immunologic responses. Women on oral contraceptives were excluded because of the enhanced DNCB responsivity associated with use of this medication ([8, 18]).

Controls for tuberculin responsivity were Mexican-born clinic and hospital patients judged to be in good general health and having neither diseases nor medications recognized as influencing immunologic responses.

DNCB responsivity was tested as previously described in detail ([22]). Briefly, a 2 mg sensitizing dose was followed in 2 to 3 weeks by challenges of 32, 16, and 8 $\mu g/cm^2$; all DNCB applications were made in a circle of 3.1 cm^2. Induration or vesciculation in an area of at least 1 cm^2 was interpreted as positive. No reaction, erythema only, or induration or vesciculation of less than 1 cm^2 was interpreted as negative. Intradermal responses to 5 tuberculin units of 5 mm or more of induration at 48 or 72 hr were interpreted as positive.

Patients' responses were compared to those of the controls by the chi-square test using the Yates' correction. Because of emphasis upon the absence of anergy, a p-value of less than 0.05 was considered to be significant.

In 8 of the untreated lepromatous subjects, the DNCB responses have been previously reported ([21]).

RESULTS

The data are summarized in the Table. Incipient reversal reactions were seen only in borderline patients. Presentation with either ENL or Lucio's reaction was seen only in lepromatous patients.

The 18 borderline and 21 lepromatous patients had a statistically significant impairment of DNCB responsivity, when compared with that of 41 controls, at each of the 3 levels tested. In contrast, the 13 tuberculoid patients had no significant impairment of DNCB responsivity.

Among the 39 borderline and lepromatous patients were 18 DNCB responders and 21 nonresponders. The 18 DNCB responders had an apparently disproportionate share of patients with reactional states—7 of the 10 with ENL, 3 of the 4 with Lucio's reaction, and 4 of the 5 incipient reversal reactions—whereas only 5 of the 21 nonresponders were troubled with reactional states. That this distribution was not a matter of chance was supported by the following analysis.

In the 19 borderline and lepromatous patients with reactional states, DNCB responsivity did not differ significantly from that of the controls. In the 20 patients without reactional states, DNCB responsivity was significantly less than that of the controls, $p < 0.0001$ at the 32 and 16 $\mu g/cm^2$ levels of responsivity.

Furthermore, among the 21 lepromatous patients, the 14 with reactional states had DNCB responsivity that did not differ significantly from that of controls, but the 7 without reactional states showed significant impairment. Similarly, among the 18 borderline patients, in the 5 with incipient reversal reactions, DNCB responsivity was

TABLE. *Responses to DNCB and PPD in numbers responding positively and (percent positive).*

Group	DNCB challenge dose			PPD	
	32μg/cm²	16μg/cm²	8μg/cm²	≥5 mm induration	≥10 mm induration
Controls[a]	35 (85)	31 (76)	22 (54)	36 (42)	32 (37)
Lepromatous (21)	11 (52)[b]	8 (38)[c]	4 (19)[b]	9 (43)	8 (38)
Borderline (18)	7 (39)[d]	6 (33)[c]	3 (17)[b]	8 (44)	7 (39)
Tuberculoid (13)	11 (85)	8 (62)	4 (31)	7 (54)	7 (54)
Borderline & lepromatous					
with reactional states (19)	14 (74)	12 (63)	5 (26)	9 (47)	8 (42)
without reactional states (20)	4 (20)[e]	2 (10)[e]	2 (10)[c]	8 (40)	7 (35)
Lepromatous					
with reactional states (14)	10 (71)	8 (57)	4 (29)	7 (50)	6 (43)
without reactional states (7)	1 (14)[d]	0 (0)[d]	0 (0)[b]	2 (29)	2 (29)
Borderline					
with reactional states (5)	4 (80)	4 (80)	1 (20)	2 (40)	2 (40)
without reactional states (13)	3 (23)[d]	2 (15)[d]	2 (15)[b]	6 (46)	5 (38)

[a] Forty-one subjects were controls for DNCB testing; 86 for PPD.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.
[e] $p < 0.0001$.

similar to controls, but in the 13 without an incipient reversal reaction, DNCB responsivity was significantly impaired.

No other groupings reciprocally responsive and unresponsive to DNCB could be identified by dividing borderline and lepromatous patients into dichotomies based upon age, sex, duration of clinical illness, extent of disease, density of bacilli per unit area of granuloma, or tuberculin responsivity.

DISCUSSION

In the present study, anergy toward DNCB was demonstrated in patients with borderline and lepromatous leprosy. This anergy toward DNCB was absent or greatly attenuated in borderline and lepromatous patients with reactional states but was strongly present in patients without reactional states, $p < 0.0001$, when compared with controls. Thus, the presence or absence of a reactional state is confirmed to be one of the variables that does explain some of the variations in the distribution of anergy in patients with leprosy. Other variables that might influence the expression of anergy—age, duration of disease, extent of disease, density of bacilli per unit of granuloma, or fortuitous environmental factors—appear to be of little or no importance in the present group of patients; our data do not bear upon the potential influence of treatment, endemicity, or genetic factors.

Only a few studies of generalized CMI responsivity in untreated patients with leprosy have been made. In 2 studies reporting anergy, reactional states were not mentioned in one ([30]) and were explicitly excluded in the other ([15]). In the study of Faber, *et al.* ([7]), reporting no anergy except with *M. leprae* as antigen, lymphocyte transformation tests were normal in untreated patients without reactional states. However, the findings of Faber, *et al.* ([7]) nevertheless are consistent with our results, i.e., the comparable *in vivo* and *in vitro* responses to tuberculin were similarly normal, but DNCB responsivity is probably not comparable to *in vitro* lymphocyte transformation tests.

Only a few studies of generalized CMI responsivity in leprosy address the question of an influence associated with reactional states. In lepromatous subjects Waldorf, *et al.* ([33]) found that 4 of 17 without ENL responded positively to DNCB, and

5 of 7 with repeated episodes of ENL were positive. In lepromatous subjects Turk and Waters [31] found that 4 of 8 with no history of ENL responded positively to DNCB and 8 of 18 with a history of ENL were positive. Hartman [11] sensitized 4 of 75 patients to DNCB in Western Kenya; 3 of those 4 had reactional states, either neuritis or ENL. Thus 2 of 3 prior studies are in accord with our findings. The most likely explanation for the discordant finding is non-comparability of patients, i.e., our ENL patients were untreated and had active ENL at the time of DNCB sensitization; those of Turk and Waters [31] were treated and had a history of ENL but evidently not active ENL at the time of sensitization.

In leprosy the requisite conditions and effector mechanisms responsible for impaired DNCB responsivity are not known. However, in the present instance, loss of responsivity to DNCB, a new antigen, but preservation of tuberculin reactivity—a coupling previously reported in leprosy [33] and in aging [34]—suggests that the DNCB unresponsiveness is not attributable to alteration in the inflammatory apparatus or to inhibition of long-lived effector T-cells.

Because impaired DNCB responsivity is strongly associated with the absence of reactional states and because reactional states are major causes of tissue injury in leprosy, impaired DNCB responsivity appears to be associated with a phenomenon of great benefit to the host, one which diminishes tissue injury. This conclusion is consistent with Kantor's [12] view of anergy in infectious diseases, i.e., an adaptive response beneficial to the host by virtue of inhibition of immunologically mediated tissue injury. Furthermore, the association of impaired DNCB responsivity with the absence of reactional states parallels the results of Bjune, *et al.* [2], who found that among borderline patients the magnitude of *M. leprae*-induced lymphocyte transformation correlated better with the clinical severity of inflammation in lesions than with the histology of the granuloma. Thus, both anergy and some types of *M. leprae*-specific CMI unresponsiveness may be associated with benefit to the host in some well-defined clinical conditions.

Several points follow from a beneficial role for anergy. In practice, drugs that might abolish the anergic state, such as levamisole, might be harmful. Also, because use of oral contraceptives enhances DNCB responsivity in normal women [8, 18], their use in women with leprosy might be associated with increased difficulties with reactional states. In theory, diminished tissue injury could give an evolutionary, selective advantage to an anergic posture, helping to explain the large numbers of conditions associated with anergy.

Recognition of a beneficial role for anergy does not exclude the possibility that anergy might also be a burden; in gaining benefit by diminished tissue injury from leprosy, is the host made more vulnerable to another illness? The question is of particular interest because it bears upon the relationship of delayed hypersensitivity and protective immunity. Three studies, citing increased incidence of basal cell carcinomas [14], lymphomas [25], and adverse reactions to vaccinia [27], suggest that anergy may be a burden for patients with leprosy. However, other explanations for those observations are plausible, and, furthermore, studies of two populations with leprosy demonstrated no significantly increased incidence of malignancies [13, 17].

This latter evidence [13, 17] indicates that if a burden exists, it is small. Our own experience is confirmatory. Thus, in our patient population, tuberculosis is the only other serious disease commonly seen, 6 of 150 patients, but in each case tuberculin responsivity has been vigorous, indicating that anergy is not a reasonable explanation for this high incidence of tuberculosis.

SUMMARY

Cell-mediated immune responses in 52 patients with untreated leprosy were measured by attempted dinitrochlorobenzene (DNCB) sensitization and tuberculin skin tests. In the 13 tuberculoid patients, DNCB responses did not differ significantly from those of the 41 controls. In the 18 borderline and 21 lepromatous patients, DNCB responses were statistically significantly less than those of the controls. Of these 39 borderline and lepromatous patients, 18 responded positively to DNCB. Included among these 18 responders were most of the patients with reactional states, i.e., 7 of 10 with erythema nodosum leprosum, 3 of

4 with Lucio's reaction, and 4 of 5 with incipient reversal reactions. Only 5 of the 20 nonresponders were so troubled with reactional states. In the 19 borderline and lepromatous patients with reactional states, DNCB responsivity did not differ significantly from that of controls. In the 20 patients without reactional states, DNCB responsivity was significantly less than that of controls, $p < 0.0001$, at the 32 $\mu g/cm^2$ and 16 $\mu g/cm^2$ levels of challenge. Thus, anergy was demonstrable in borderline and lepromatous subjects, was absent or greatly attenuated in reactional states, and was particularly prevalent in patients without reactional states. Because reactional states are a major source of tissue injury in patients with leprosy, anergy is, or is associated with, a phenomenon of great benefit to the host.

RESÚMEN

Se estudió la respuesta inmune celular en 52 pacientes con lepra no tratada en base a su capacidad para sensibilizarse al dinitroclorobenceno (DNCB) y a su reactividad a la tuberculina. En los 13 pacientes tuberculoides, las respuestas al DNCB no difirieron significativamente de las respuestas de los 41 controles. En los 18 pacientes con lepra intermedia ("borderline") y en los 21 lepromatosos, las respuestas al DNCB fueron significativamente menores que las de los controles. De estos 39 pacientes "borderline" y lepromatosos, 18 respondieron positivamente al DNCB. Entre los 18 que respondieron estuvieron incluídos la mayoría de los pacientes con reacción leprosa, es decir, 7 de 10 con eritema nodoso leproso, 3 de 4 con reacción de Lucio, y 4 de 5 con reacciones reversas incipientes. Sólo 5 de los 20 que no respondieron presentaron reacción leprosa. En los 19 pacientes "borderline" y lepromatosos con reacción leprosa, la frecuencia de respuestas al DNCB fueigual a la frequencia de los controles. En los 20 pacientes sin reacción, la frequencia de respuestas al DNCB fue significativamente menor que la de los controles, $p < 0.0001$, a las dosis de reto de 32 $\mu g/cm^2$ y de 16 $\mu g/cm^2$. Resumiendo, la anérgia fue demostrable en los pacientes "borderline" y lepromatosos, estuvo ausente o fue muy poco aparente en los pacientes con reacción leprosa, y fue particularmente prevalente en los pacientes sin reacción leprosa. Debido a que los estados reaccionales son una causa muy importante de daño tisular en los pacientes con lepra, la anérgia es, o está asociada con, un fenómeno de gran beneficio para el paciente.

RÉSUMÉ

Chez 52 malades atteints de lèpre non traitée, on a mesuré les réponses immunitaires dépendant de la médiation cellulaire, au moyen d'essais de sensibilisation au dinitrochlorobenzène (DNCB), et par des épreuves cutanées à la tuberculine. Chez 13 malades tuberculoïdes, les réponses au DNCB n'ont pas différé significativement de celles enregistrées chez 41 témoins. Chez 18 malades borderline, et chez 21 lépromateux, les réponses au DNCB étaient moindre que celles exhibées par les témoins, et ceci de façon statistiquement significative. Parmi les 39 malades borderline et lépromateux, 18 ont répondu positivement au DNCB. Parmi ces 18 malades répondant positivement, se trouvait la plupart des malades présentant un état réactionnel, à savoir 7 sur les 10 avec un érythème noueux lépreux, 3 sur les 4 qui présentaient une réaction de Lucio, et 4 sur les 5 commençant une réaction inversée (reversal reaction). Cinq des 20 malades qui ne répondaient pas au DNCB, seulement, présentaient de tels états réactionnels. Parmi les 19 sujets atteints de lèpre borderline ou lépromateuse avec réaction, la capacité de réponse au DNCB ne différait pas significativement de celle observée chez les témoins. Chez les 20 malades sans état de réaction, la capacité de réponse au DNCB était significativement moindre que chez les contrôles, au taux de stimulation de 32 $\mu g/cm^2$ et de $\mu g/cm^2$ ($p < 0,0001$). On pouvait démontrer cette allergie chez les sujets lépromateux et chez les sujets borderline; cette allergie était absente ou fortement atténuée dans les états de réaction; elle était particulièrement fréquente chez les malades sans réaction. Les états réactionnels constituant une cause importante de lésions tissulaires chez les malades atteints de lèpre, l'anergie est, ou tout au moins est associée à, un phénomène fort avantageux pour le malade.

REFERENCES

1. Barnetson, R. St. C., Pearson, J. M. H. and Rees, R. J. W. Evidence for prevention of borderline reactions by dapsone. Lancet **2** (1976) 1171–1172.
2. Bjune, G., Barnetson, R. St. C., Ridley, D. S. and Kronvall, G. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. Clin. Exp. Immunol. **25** (1976) 85–94.
3. Bullock, W. E. Studies of immune mechanisms in leprosy. I. Depression of delayed allergic responses to skin test antigens. N. Engl. J. Med. **278** (1968) 298–304.
4. Convit, J., Pinardi, M. E. and Rojas, F. A. Some considerations regarding the immunology of leprosy. Int. J. Lepr. **39** (1971) 556–564.
5. Dierks, R. E. and Shepard, C. C. Effect of phytohemagglutinin and various mycobacterial antigens on lymphocyte cultures from leprosy patients. Proc. Soc. Exp. Biol. Med. **127** (1968) 391–395.
6. Dwyer, J. M., Bullock, W. E. and Fields, J. P. Disturbance of the blood T:B lymphocyte ratio in lepromatous leprosy. Clinical and immunologic

correlations. N. Engl. J. Med. **288** (1973) 1036–1039.

7. Faber, W. R., Leiker, D. L., Nengerman, I. M., Zeijlemaker, W. P. and Schellekens, P. Th. A. Lymphocyte transformation test in leprosy: decreased lymphocyte reactivity to *Mycobacterium leprae* in lepromatous leprosy with no evidence for a generalized impairment. Infect. Immun. **22** (1978) 649–656.
8. Gerretsen, G., Kremer, J., Bleumink, E. and Nater, P. J. Dinitrochlorobenzene sensitisation test in women on hormonal contraceptives. Lancet **2** (1975) 347–349.
9. Han, S. H., Weiser, R. S. and Kau, S. T. Prolonged survival of skin allografts in leprosy patients. Int. J. Lepr. **39** (1971) 1–6.
10. Han, S. H., Weiser, R. S and Lin, Y. C. Transformation of leprous lymphocytes by leprolin, tuberculin and phytohemagglutinin. Int. J. Lepr. **39** (1971) 789–795.
11. Hartman, A. DNCB-reactivity in patients with leprosy in Kenya. Lepr. Rev. **47** (1976) 193–199.
12. Kantor, F. S. Infection, anergy and cell-mediated immunity. N. Engl. J. Med. **292** (1975) 629–634.
13. Kolonel, L. N. and Hirohata, T. Leprosy and cancer: a retrospective cohort study in Hawaii. J. Natl. Cancer Inst. **58** (1977) 1577–1581.
14. Michalany, J. Malignant tumors of the skin among leprosy patients. Int. J. Lepr. **34** (1966) 274–286.
15. Nath, I., Curtis, J., Bhutani, L. K. and Talwar, G. P. Reduction in a subpopulation of T lymphocytes in lepromatous leprosy. Clin. Exp. Immunol. **18** (1974) 81–87.
16. Nelson, D. S., Nelson, M., Thurston, J. M., Waters, M. F. R. and Pearson, J. M. H. Phytohaemagglutinin-induced lymphocyte transformation in leprosy. Clin. Exp. Immunol. **9** (1971) 33–43.
17. Oleinick, A. Altered immunity and cancer risk: a review of the problem and analysis of the cancer mortality experience of leprosy patients. J. Natl. Cancer Inst. **43** (1969) 775–781.
18. Rea, T. H. Quantitative enhancement of dinitrochlorobenzene responsivity in women receiving oral contraceptives. Arch. Dermatol. **115** (1979) 361–362.
19. Rea, T. H. and Levan, N. E. Erythema nodosum leprosum in a general hospital. Arch. Dermatol. **111** (1975) 1575–1580.
20. Rea, T. H. and Levan, N. E. Lucio's phenomenon and diffuse nonnodular lepromatous leprosy. Arch. Dermatol. **114** (1978) 1023–1028.
21. Rea, T. H., Quismorio, F. P., Harding, B., Friou, G. and Levan, N. Quantitative dinitrochlorobenzene (DNCB) responsivity and phytohemagglutinin (PHA) induced lymphocyte transformation in patients with lepromatous leprosy. Int. J. Lepr. **44** (1976) 250–255.
22. Rea, T. H., Quismorio, F. P., Harding, B., Neis, K. M. Di Saia, P. J., Levan, N. E. and Friou, G. J. Immunologic responses in patients with lepromatous leprosy. Arch. Dermatol. **112** (1976) 791–800.
23. Ridley, D. S. Reactions in leprosy. Lepr. Rev. **40** (1969) 77–81.
24. Ridley, D. S. Histological classification and the immunological spectrum of leprosy. Bull. WHO **51** (1974) 451–465.
25. Rodriquez, E., De Bonaparte, Y. P., Morgenfeld, M. C. and Cabrini, R. L. Malignant lymphomas in leprosy patients. A clinical and histologic study. Int. J. Lepr. **36** (1968) 203–212.
26. Saha, K. and Agarwal, S. K. Immune deficit in patients with lepromatous leprosy: Its nature and relation to genetic factors, spectrum and duration of illness. Int. J. Lepr. **47** (1979) 1–6.
27. Saha, K. and Mittal, M. M. Consequences of smallpox vaccination in leprosy patients. Infect. Immun. **8** (1973) 301–308.
28. Salazar-Mallen, M., Zamora, A. C., Montes, J. M., Escobar, A., Amezcua, M. E. and Levy, D. M. Estudios sobre la immunidad de la lepra. Alergia **18** (1971) 185–191.
29. Sheagren, J. N., Block, J. B., Trautman, J. R. and Wolff, M. Immunologic reactivity in patients with leprosy. Ann. Intern. Med. **70** (1969) 295–302.
30. Talwar, G. P., Krishnan, A. D., Mehra, V. L., Blum, E. A. and Pearson, J. M. H. Evaluation of cell mediated immune responses in untreated cases of leprosy. Clin. Exp. Immunol. **12** (1972) 195–203.
31. Turk, J. L. and Waters, M. F. R. Cell-mediated immunity in patients with leprosy. Lancet **2** (1969) 243–246.
32. Ulrich, M., De Salas, B. and Convit, J. Lymphocyte transformation with phytomitogens in leprosy. Int. J. Lepr. **40** (1972) 4–9.
33. Waldorf, D. S., Sheagren, J. N., Trautman, J. R and Block, J. B. Impaired delayed hypersensitivity in patients with lepromatous leprosy. Lancet **2** (1966) 773–776.
34. Waldorf, D. S., Willkens, R. F. and Decker, J. L. Impaired delayed hypersensitivity in an aging population. J. Am. Med. Assoc. **203** (1968) 831–834.