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An HLA-linked gene controls susceptibility to lepromatous leprosy through T cell regulation

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Abstract
Genetic control of the clinical manifestation of leprosy was investigated in 66 unrelated patients with leprosy and 8 multiplex families. In 32 lepromatous leprosy(LL) patients, both phenotype frequency of HLA-DR2 and haplotype frequency of HLA-B35-DR2-DQwl were significantly increased. Our family data combined with other investigators' showed that the distribution of shared HLA haplotypes differed significantly from the random distribution, thereby suggesting the existence of an HLA-linked major gene for lepromatous leprosy. To investigate the function of this major gene, the cellular mechanism of nonresponsiveness of LL that is strictly specific to *mycobacterium leprae* (ML) antigen was analysed using panning technique and monoclonal antibodies. We have tested 30 LL patients for their suppressive activity of T8 cells on the T cell response to ML of tuberculoid leprosy(TT) patients. T8 cells from two LL patients abrogated the response of TT patients. None of LL patients tested showed proliferative response to ML antigen even when we removed the T8 cells from the culture. Therefore, we concluded that nonresponsiveness to ML antigen of LL patients in vitro were generated by the elimination of responding T cells. In the minor population of LL, T8 suppressor T cells were still active in peripheral blood. The T8 suppressor T cells might play some role in the elimination of responding T cells to ML antigen.

Results and discussion
As shown in Table 1, the frequency of both HLA-DR2 antigen and HLA-B35-DR2-DQwl haplotype was significantly increased in 32 unrelated LL patients. The frequency of this haplotype was not increased in tuberculoid patients. Recently in narcolepsy patients, B35-DR2-Dw2-DQwl haplotype was strikingly increased by the study of Juji et al (1). HLA-D typing of LL patients is now under investigation. For the second step, we have performed linkage analysis using affected sib pair method (2) as shown in table 2. Out of 8 affected sib pairs with LL, 2 sib pairs could be assigned as sharing two HLA haplotypes identical by descent, 6 sib pairs could be assigned as sharing one haplotype and none could be assigned as sharing no haplotype. Our data were combined with other investigators' results. The HLA haplotype distribution differed significantly from the random expected ($X^2 = 8.25$, D.F. $= 2$, $p < 0.02$), thereby suggesting the existence of an HLA-linked major gene for lepromatous leprosy.

To investigate the function of this major gene, we analysed the nonresponsiveness to ML antigen of LL patients. The immune responsiveness was measured by the T lymphoproliferative response of peripheral blood lymphocytes (PBL) specific for soluble antigen in vitro. PBL from LL patients showed no response to ML antigen, whereas they showed good response to other nominal antigens, such as purified protein derivatives (PPD), streptococcal cell wall antigen and candida albicans antigen (data not shown). These observations indicated that the nonresponsiveness to ML antigen of LL patients was antigen-specific.

Table 1.
Association between HLA and lepromatous leprosy.

HLA[a]	Patients[b] (N=32)	Controls (N=95)	Relative risk	X^2	Pc[c]
Bw52	7.5 %	20.0 %	0.32	2.37	n.s.[d]
B35	27.3	14.0	2.36	2.89	n.s.
DR2	59.4	29.0	3.57	8.97	0.05
DQwl	78.1	63.9	2.01	1.71	n.s.
Bw52-DR2-DQwl	7.81[e]	9.57	0.81	0.02	n.s.
B35-DR2-DQwl	12.50	1.63	9.23	11.78	0.04

a. HLA-A,B and C typing was performed using an NIH standard microcytotoxicity technique.
HLA-DR and DQ typing was performed according to the method used in the 8th International Histocompatibility Workshop and Conference. Typing reagents used in this study were 282 anti HLA ABC and 165 HLA-DR, DQ sera distributed for the second Asia-Oceania Histocompatibility Workshop.
b. The patients were diagnosed clinically and histologically according to the immunological spectrum described by Ridley and Jopling (3). 32 Unrelated LL patients (21 males and 11 females) were all from main islands of Japan.
c. Pc, corrected P.
d. n.s., not significant
e. haplotype frequency

Table 2.
Mode of inheritance and linkage analysis by the affected sib pairs.

Shared HLA haplotypes	Present study	van Eden et al (4)	Others (5,6,7)	Obs.[a] Total	Exp. $(X^2)^b$
Two	2	8	2	12	6.00(6.00)
One	6	1	2	9	12.00(0.75)
None	0	2	1	3	6.00(1.50)
Total	8	11	5	24	(8.25)[c]

a. Obs; observed number, Exp; expected number
b. Based on random ratio 1:2:1
c. D.F.=2, $p<0.02$

Furthermore, Figure 1 shows that the nonresponsiveness of LL was determined by T cells. The monocyte of LL presented ML antigen to T cells of TT patients. The T cells of LL did not show any response even in the presence of HLA-DR matched monocytes of TT.

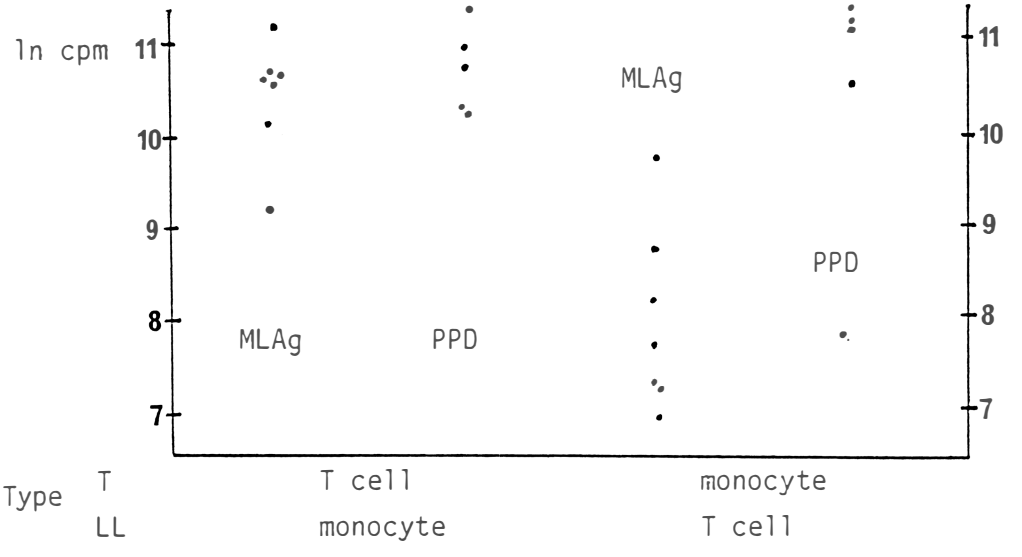

Figure 1.
Antigen specific nonresponsiveness of LL was determined by T cells, not by monocytes.

$1x10^5$ T cells and $0.1x10^5$ to $0.05x10^5$ allogenic monocytes were cocultured in flat bottomed microtiter plates in the presence of 0.5μg of MLAg or 5.0μg of PPD for 7 days.

We considered that the T cell dysfunction of LL was generated by following two alternatives. One is the active suppression and the other is the clonal deletion.

Therefore, we have tested 30 LL patients for their suppressor activity of T8 cells or for their responsiveness of T4 cells. Out of 30 LL patients, 2 patients were positive for their suppressor activity of T8 cells (Table 3). The immune response of PBL to ML antigen of HS (TT) (cpm = 50.982) was abrogated by the addition of T cells or T8 cells of SK (LL) (96.5 ~ 100 % suppression). The other LL patients showed no response to ML antigen, even when we removed T8 cells from the culture.

From these observations we concluded that nonresponsiveness to ML antigen of LL patients in vitro were generated by the elimination of responding T cells, but that in the minor population of LL, T8 suppressor T cells were still active in peripheral blood.

The T8 suppressor T cells might play some role in the elimination of responding T cells to ML antigen during the long clinical course of LL. Further characterization of the suppression of T8 cells of LL including suppressor factor and the role of HLA is now under investigation.

Table 3.
T8 suppressor T cells of LL abrogated the immune response specific for ML antigen of TT.

Cells	ML	Med	Δcpm (% supp.)[b]
HS (TT) PBL [a]	50,982	1,383	49,599
+SK (LL) T cells	6,323	4,621	1,702 (96.6)
+SK T8	749	3,587	–2,838 (100.0)
+SK T4	134,853	5,180	129,673 (–161.4)
SK (LL) PBL	5,266	2,191	3,075

a. Peripheral blood lymphocytes (PBL) ($1x10^5$) from TT patients (HS) were co-cultured with LL T cells ($2x10^5$), T8 cells or T4 cells ($1x10^5$) from LL patients (SK) in the presence of 0.5 /ml MLAg for 7 days. T cell subsets were separated by the panning method using anti Leu 2a monoclonal antibody (8).
The HLA type of the patients were
HS: A2, – B35,w52, Cw3,–,DR2,– DQwl, –.
SK: A2,24, B35,w52, Cw3,w7, DR2,4, DQwl, –.
b. % suppression was calculate by the following formula:

$$\% \text{ suppression} = \frac{\Delta\text{cpm of responder} - \Delta\text{cpm of test sample}}{\Delta\text{cpm of responder}} \times 100$$

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