

LYMPHOCYTE ABNORMALITY ASSOCIATED WITH
HLA-B8 IN HEALTHY YOUNG ADULTS*

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One of the most important developments in clinical immunology in the past decade has been the demonstration of increased frequencies of specific histocompatibility antigens in individuals with a variety of diseases, implying that genes predisposing to the disorders reside in or near the major histocompatibility complex. The increased prevalence of the antigens HLA-B8, HLA-Dw3, and HLA-DRw3 in a number of organ-specific autoimmune diseases suggests that a gene in the HLA region, associated with these alleles, may predispose to the development of autoimmunity. One of the HLA-B8-associated diseases is Sjogren's syndrome, an autoimmune disorder characterized by lymphocytic infiltrates of salivary, lacrimal, and other exocrine glands.

We reported (1) an impaired lymphocyte response to suboptimally stimulating concentrations of phytohemagglutinin (PHA) in Sjogren's syndrome. This abnormality of lymphocyte activation occurs almost exclusively in patients with HLA-B8 (2), and we hypothesized that it might reflect the action of a gene predisposing to autoimmunity.

To elucidate the possible etiological significance of this lymphocyte abnormality in Sjogren's syndrome, we examined the lymphocyte responses of healthy young adults with or without HLA-B8. We now report that most normal subjects with HLA-B8 have a similar impairment of lymphocyte activation in response to suboptimal concentrations of PHA. The decreased proliferation is also apparent in the response to suboptimal concentrations of concanavalin A (Con A) but not to optimally stimulating concentrations of either mitogen. Subjects with HLA-B8 had similar percentages of T lymphocytes, and the abnormality could be demonstrated in highly purified suspensions of T cells.

Materials and Methods

Subjects. Clinically healthy Caucasian college students were typed for HLA antigens using trays purchased from Dr. Paul Terasaki, UCLA, Los Angeles, CA. All subjects were negative for rheumatoid factor and anti-nuclear antibodies. To minimize experiment-to-experiment variability, the following protocol was used for assays of mitogen responsiveness. Two or three individuals with HLA-B8, an equal number of age- and sex-matched controls, and an additional reference individual were included in each experiment. The results of six experiments were combined and the data normalized, based upon the response of the reference individual, who was included in all experiments.

HLA Typing. HLA antigens of the A, B, C, and DR loci were detected by the microlym-

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phocyte cytotoxicity method (3) using typing trays purchased from Dr. Terasaki. Rabbit complement, prescreened for effectiveness and lack of toxicity, was obtained from Accurate Chemical and Scientific Co., Westbury, NY. For HLA-DR typing, B lymphocytes were recovered from soda-straw nylon wool columns (4).

Cell Preparation. Human peripheral blood was obtained by venipuncture from normal subjects. Mononuclear cells were separated from heparinized blood by centrifugation on Ficoll-Hypaque gradients (5). Cells were washed three times before being placed in culture.

DNA synthesis in response to mitogens was measured as previously described (6). Final concentrations of PHA used included 0.05 $\mu\text{g/ml}$, 0.075 $\mu\text{g/ml}$, 0.1 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$, and 1.0 $\mu\text{g/ml}$. Final concentrations of Con A included 0.5 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$, 3.3 $\mu\text{g/ml}$, and 10 $\mu\text{g/ml}$. For most subjects, 1.0 $\mu\text{g/ml}$ of PHA and 10 $\mu\text{g/ml}$ of Con A resulted in an optimal proliferative response.

Cell Separation and Identification. Sephadex G-10 bead columns were prepared according to the method of Ly and Mishell (7), with the following modifications (8). Columns were prepared in 12-ml syringes, plugged with a small amount of glass wool. 9 ml of Sephadex beads were added to each column. Up to 1×10^8 peripheral blood mononuclear cells were added to the column in 1 ml of RPMI with 5% fetal calf serum (FCS). Columns were incubated at 37°C for 30 min and stirred gently with a pipette at 10-min intervals. After incubation, nonadherent cells were flushed from the column in 25–30 ml of RPMI with FCS.

T lymphocytes were purified by passage over nylon wool columns, according to the method of Julius (9), except that a preliminary passage over Sephadex columns was substituted for preliminary passage over glass wool columns. T lymphocytes capable of forming rosettes with sheep erythrocytes were identified by the method of Jondal (10). Peripheral blood mononuclear cells were mixed with washed sheep erythrocytes at a ratio of 1:200. The cells were pelleted and allowed to sit at room temperature overnight. After gentle resuspension, lymphocytes binding three or more erythrocytes were counted. Monocytes were identified by staining slides prepared in a Shandon cytocentrifuge for myeloperoxidase (11, 12). Cells recovered from such a double-column passage contained ~1% monocytes and 0.3% B cells (by staining for surface immunoglobulin (Ig)).

Statistical Analysis. The data presented are the means of quadruplicate cultures after subtraction of unstimulated (background) incorporation. In analyzing proliferative responses, geometric means were used to reflect the logarithmic nature of the data.

Results

Mitogen Responses. When peripheral blood mononuclear cells from clinically normal young adults with and without HLA-B8 were cultured with suboptimally stimulating concentrations of PHA, most of the subjects with HLA-B8 demonstrated an impaired proliferative response. In response to 0.1 $\mu\text{g/ml}$ of PHA, lymphocytes from 9 out of 15 HLA-B8-positive subjects incorporated <2,500 cpm, compared with only 1 out of 15 subjects without HLA-B8 (Fig. 1A). The mean response of the subjects with HLA-B8 was 1,919 cpm, significantly less than the response (6,384 cpm) of the subjects without HLA-B8 ($P < 0.007$). This concentration of PHA is ~10% of the optimal concentration.

Many of the healthy subjects with HLA-B8 also had a low response to a suboptimally stimulating concentration (0.5 $\mu\text{g/ml}$) of the mitogen Con A (Fig. 1B). The mean response of the HLA-B8 group (5,559 cpm) was significantly less than the mean response (11,550 cpm) of healthy subjects without this antigen ($P < 0.009$). Of the nine HLA-B8-positive subjects with suboptimal PHA responses below 2,500 cpm, seven also had suboptimal Con A responses below 4,000 cpm. Lymphocyte proliferation in response to an optimally stimulating concentration of PHA (1.0 $\mu\text{g/ml}$) did not differ significantly in the two groups.

Subjects with HLA-B8 tended to have somewhat lower responses to an optimally stimulating concentration of Con A (10 $\mu\text{g/ml}$) than the HLA-B8-negative subjects,

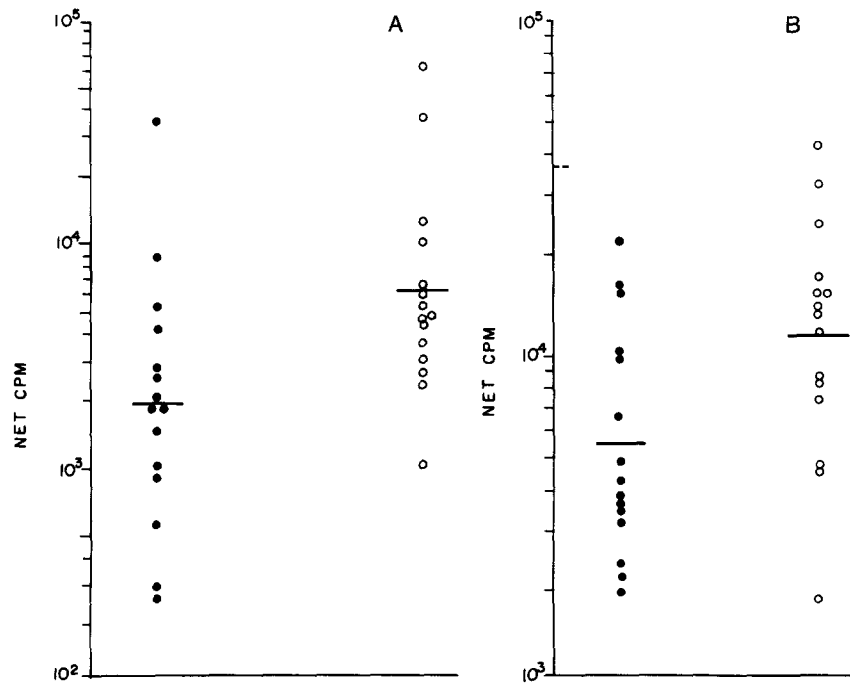


FIG. 1. Lymphocyte responses of clinically normal subjects with (●) or without (○) HLA-B8. Bars indicate geometric means. (A) response to 0.1 $\mu\text{g/ml}$ PHA. HLA-B8-positive subjects; mean = 3.283 ± 0.1432 . HLA-B8-negative subjects; mean = 3.805 ± 0.1315 ; $t = 2.648$; $P < 0.007$. (B) response to 0.5 $\mu\text{g/ml}$ Con A. HLA-B8-positive subjects; mean = 3.745 ± 0.0861 . HLA-B8-negative subjects; mean = 4.062 ± 0.0904 . $t = 2.543$; $P < 0.009$.

but the difference between the two groups was not significant ($P < 0.07$). Of the nine subjects with HLA-B8, who had optimal Con A responses below 60,000 cpm, eight of these individuals also had suboptimal PHA responses below 2,500.

HLA-DR Typing. HLA antigens of the DR locus were determined for 11 clinically normal subjects with the antigen HLA-B8. Of the 11 HLA-B8-positive subjects typed, 9 also had the associated antigen DRw3. Of the two subjects without HLA-DRw3, one had a response to suboptimal PHA of 2,812 cpm, just above the mean of the HLA-B8 group. The other subject without DRw3 had a suboptimal PHA response of 263 cpm, the lowest response in the study.

Percentages of T Cells and Monocytes in Peripheral Blood of Normal Subjects with or without HLA-B8. After isolation of mononuclear cells on Ficoll-Hypaque gradients, an average of 1.18×10^7 cells per 10 ml of blood was recovered from subjects with HLA-B8, compared with 1.77×10^7 cells per 10 ml of blood from subjects without this antigen. The lower cell recovery from blood from HLA-B8 subjects was not due to a decreased percentage of T lymphocytes in HLA-B8 subjects, as 56.9% of the mononuclear cells formed rosettes, compared with 57.0% of cells from subjects without HLA-B8. Significantly fewer peroxidase-positive monocytes were observed on cytopsin slides from HLA-B8-positive subjects (24.8%), compared with subjects without this antigen (39.5%).

Functional Impairment of T Lymphocytes. In experiments designed to determine the cellular basis of the abnormal PHA response, T lymphocytes were purified by sequential passage over Sephadex G-10 and nylon wool columns. Three experiments

TABLE I
Impaired PHA Response of Subjects with HLA-B8 Is Attributable to T Lymphocytes

| Experiment | Cell preparation | Normal subject with HLA-B8 | Percent per-oxidase staining monocytes | Normal subject without HLA-B8 | Percent per-oxidase staining monocytes |
|------------|-------------------|----------------------------|--|-------------------------------|--|
| 1 | Unseparated cells | 3,445 ± 242* | (13.3%) | 14,065 ± 204 | (28.9%) |
| | T lymphocytes‡ | 1,648 ± 327 | (2.0%) | 6,737 ± 316 | (2.0%) |
| 2 | Unseparated cells | 2,982 ± 134 | (11.5%) | 8,468 ± 303 | (21.8%) |
| | T lymphocytes | 533 ± 67 | (0.3%) | 2,802 ± 236 | (0.5%) |
| 3 | Unseparated cells | 9,396 ± 1651 | (17.5%) | 33,245 ± 1,081 | (17.5%) |
| | T lymphocytes | 572 ± 21 | (0.6%) | 14,185 ± 1,413 | (1.0%) |

* Incorporation of tritiated thymidine in response to 0.1 µg/ml PHA. Results expressed as net cpm ± SE.

‡ T lymphocytes were purified by passage over Sephadex G-10 columns followed by passage over nylon wool columns.

using this protocol are shown in Table I. Highly purified T cells proliferated less in response to a suboptimal concentration of PHA than did the original cell suspensions, but when subjects with or without HLA-B8 were compared, in each case the purified T cells from the subjects with HLA-B8 retained the same impaired response relative to the non-HLA-B8 subjects.

Discussion

The mechanism of genetic predisposition to autoimmune disorders is unknown, but may involve HLA-linked immune response genes, susceptibility to infectious agents, or the action of other genes in the HLA region that are involved in immune functions. Several previous studies as well as our own results suggest that a gene associated with HLA-B8 may act through an effect on the immune system that is detectable in clinically normal individuals.

Lymphocyte proliferation in vitro to wheat gluten has been reported to be significantly more frequent in HLA-B8-positive normal subjects than in those without this antigen (13). Among patients with skin cancer, a significant correlation of HLA-B8 with large tumors and with low T cell levels has been reported (14). A study by Greenburg and Yunis (15) found a decreased PHA response by lymphocytes from normal women with HLA-B8.

Lawley and associates (16) recently reported that normal subjects with the HLA-B8/DRw3 haplotype had delayed Fc receptor-mediated clearance of IgG-sensitized erythrocytes, decreased percentages and total numbers of T cells bearing Fc receptors for IgG, decreased Con A responses, and increased numbers of circulating spontaneous Ig-secreting cells.

Ambinder and associates (17) recently reported that lymphocytes from normal subjects with HLA-DR3 developed increased numbers of plaque-forming cells in culture and have decreased Con A-induced suppression.

Our results indicate that healthy young adults with the antigen HLA-B8 have an abnormality of lymphocyte function that is expressed in vitro as a decreased ability to proliferate in response to suboptimal concentrations of PHA and Con A. The fact that the lymphocyte abnormality is a discrete defect of response to suboptimal concentrations of PHA, while the response to higher concentrations is normal, offers a clue to the possible mechanism. The response to suboptimal mitogen concentrations differs from the response of optimal concentrations in several respects.

First, the response to a suboptimal concentration of PHA is highly dependent on cellular interactions, whereas the response to optimal concentrations is not. Oppenheim and associates (18) as well as ourselves (6) have shown that depleting monocytes

or macrophages decreases the response to suboptimal concentrations far more than optimal responses.

Second, the cells proliferating in response to suboptimal concentrations of mitogens may represent a distinct T cell subpopulation. Schlossman and his associates (19) have shown that only the OKT4+ subpopulation responds to suboptimal concentrations of PHA.

A third difference between suboptimum and optimum responses is that suboptimal mitogen responses are far more sensitive to inhibition by physiological concentrations of hydrocortisone, histamine, interferon, isoproterenol, and prostaglandins (20, 21). The reason for the differential sensitivity of suboptimal responses to these agents is unclear, but it appears that optimal concentrations of mitogens bypass or override regulatory mechanisms.

The results of cell separation experiments suggest that the defect of lymphocyte activation is intrinsic to the T lymphocytes because purified T cells from HLA-B8-positive subjects show the same relative impairment compared with T cells from subjects without this antigen. Although we found no difference in the total numbers of T lymphocytes, a qualitative or quantitative abnormality of a T lymphocyte subpopulation remains a possibility.

Like other investigators, we used HLA-B8 as a convenient marker gene. We presume that the gene responsible for the lymphocyte defect, like the gene thought to predispose to autoimmunity, is in linkage disequilibrium with HLA-B8, Dw3, and DRw3. The fact that some individuals with HLA-B8 have responses to suboptimal mitogen concentrations well within the range of individuals without this antigen, confirms that the gene responsible is not HLA-B8 per se. The finding that one of two HLA-B8-positive subjects in this study, who lacked HLA-DRw3, also has a greatly impaired response, suggests that the responsible gene is also not DRw3 per se.

Our observation of a defect of lymphocyte activation associated with HLA-B8 patients with Sjogren's syndrome led us to hypothesize that this abnormality might directly reflect the action of the gene associated with HLA-B8, which predisposes to autoimmunity. Our demonstration that many healthy young adults with HLA-B8 have a similar abnormality indicates that the lymphocyte defect has a direct genetic basis, precedes the development of autoimmunity, and might, therefore, be of etiological significance. Further analysis of this *in vitro* abnormality might help to elucidate the mode of action of the responsible gene.

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Summary

We have reported that abnormal lymphocyte function in Sjogren's syndrome occurs almost exclusively in patients with HLA-B8. We now report that most clinically normal individuals with this antigen have a similar impairment of cellular immunity. This finding suggests that the lymphocyte abnormality in Sjogren's syndrome is not secondary to the disease process or medication and might have primary etiological significance. The lymphocyte abnormality is expressed as a decreased proliferative response to suboptimally stimulating concentrations of phytohemagglutinin (PHA) and concanavalin A (Con A). In contrast, the response to optimally stimulating concentrations of PHA and Con A is unaffected. The impaired mitogen responsiveness appears to be intrinsic to the T lymphocytes, as it can be demonstrated in purified T cell preparations.

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