fields. The second of the above requirements, demanding differential values in $\ell$ and $D/U$, must therefore be met entirely by differences in $D$. Careful considerations show that this requirement is not a disadvantage, and may be useful in correlating retention data with $D$ and in measuring $D$.

We constructed an apparatus to test this concept. We formed the channel by clamping two membranes together over a stainless steel spacer 0.0254 cm thick from which a flow space 1 by 45 cm had been cut. The upper and lower membranes were cellulose acetate and Amicon UM-20 membrane, respectively. The two flow streams were supplied by metering pumps (Laboratory Data Control model CMP IV). Samples containing about 20 $\mu$g of solute were injected at the flow inlet, and the eluted peaks were detected with an ultraviolet detector (Laboratory Data Control). A slightly modified system was used for fractionation.

Positive retention was observed. Furthermore, retention varied from solute to solute, demonstrating a basic fractionating capability of the system.

In order to determine if the retention parameters depend on $D$ in the predicted way, we used the relationships

$$\lambda = \ell/w$$

$$|U| = V_c/aL$$

and

$$V_0 = aLw$$

along with Eq. 1 to derive

$$\frac{\lambda \dot{V}_c V_0}{a^2 L^2} = D \qquad (3)$$

where $\dot{V}_c$ is the volumetric rate of cross flow, $V_0$ is the channel volume, $a$ is its width, and $L$ is its length. We then plotted the left-hand term of Eq. 3, which depends on observed retention through $\lambda$, against independent values of $D$. For polystyrene beads, we calculated $D$ from the Stokes-Einstein equation; for proteins and viruses we used literature values (*5*). The results are shown in Fig. 2, where the solid line represents the prediction of Eq. 3. The data are thus in basic accord with theory.

The solutes tested here represent an enormous size range, from polystyrene beads with a mean diameter of 4810 Å to $\beta$-casein with a molecular weight of 24,100. The mass ratio between these extremes is about 1.5 million. The potential range is even greater as there are no theoretical limits, only practical ones.

An example of fractionation by flow FFF is shown in Fig. 3. We have achieved a resolution or partial resolution of six protein components. Theory suggests that a much better resolution between peaks is possible, but the implementation of this prospect will require additional studies.

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## Multiple Sclerosis and High Incidence of a B Lymphocyte Antigen

Abstract. *Multiple sclerosis patients were tested for six new antigens present on human B lymphocytes. The group 4 specificity occurred in 83.9 percent of the 56 patients as compared to 32.5 percent in 72 healthy controls ($P < .003$). The antiserums defining the five other B lymphocyte specificities reacted at a lower frequency to B cells from multiple sclerosis patients, showing that increased reactivity to group 4 antiserums was specific. Linkage of a hypothesized multiple sclerosis susceptibility gene with certain haplotypes of HLA-A3, HLA-B7, HLA-DW2, and the new B group 4 can be inferred.*

We initially suggested the possibility that the HLA region would serve as a genetic marker for susceptibility to multiple sclerosis (MS) by finding that HLA-A3, an A locus antigen, was of higher frequency among MS patients (*1*). This was confirmed by others (*2*), and in addition a B locus antigen, HLA-B7, was shown to be highly associated with MS (*2*). It was next shown that the D locus antigen, DW2 (LD7a), had a higher association with MS (*3*). The inference drawn was that the MS susceptibility gene is probably closer to the D locus than the B or A loci of HLA (*3*). Winchester *et al.* have reported finding a single serum that reacted with the B lymphocytes from 25 MS patients tested (*4*).

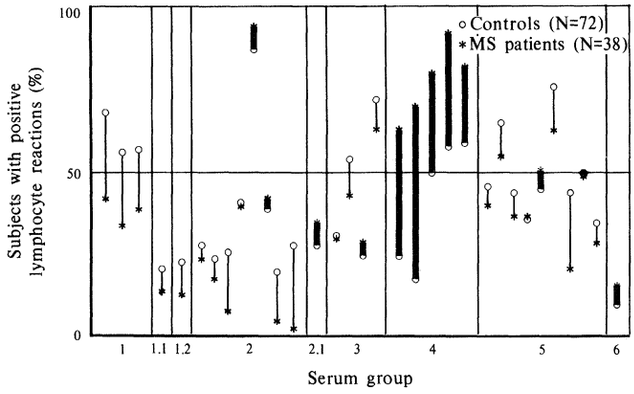
B lymphocytes have been shown to have antigens that are distinct from HLA (*5, 6*). The antiserums that react with B lymphocytes but not T lymphocytes have been grouped with respect to their antigenic specificities (*6*). We now describe the typing of B lymphocytes from MS patients for six specificities and the unusually high frequency of group 4 antigen in this disease.

Several centers sent blood from 56 Caucasian MS patients to our laboratory (*7*). Lymphocytes were isolated within 15 hours with the Ficoll-Hypaque density technique and stored in vials in liquid nitrogen. Lymphocytes from 72 healthy control Caucasians were similarly stored in vials in liquid nitrogen. Since HLA frequencies are principally affected by race (and not by age, sex, state of health, or the like), the main effort in matching controls to patients was by restriction to Caucasians.

The B lymphocytes were isolated from blood by removal of T cells by rosetting with neuraminidase-treated sheep red blood cells and centrifugation through Ficoll-Hypaque. These cells were reacted for 30 minutes with a panel of antiserums. After a further 3-hour incubation with rabbit complement, the cells were stained with eosin and fixed with formaldehyde.

The antiserums were selected from more than 400 serums of parous women or patients who had undergone transfusions. The serums were absorbed twice with pooled, packed platelets until all activity against T lymphocytes had been removed. From analysis of the reactions, 33 serums that reacted as six separate specificities were selected (*6*). The HLA-AB locus typing and HLA-DW2 typing were performed by standard methods.

The frequency of reactivity of 33 serums against B lymphocytes of the first 38 MS patients and against controls is given in Fig. 1. The bars represent the difference in frequency of reactivity be-



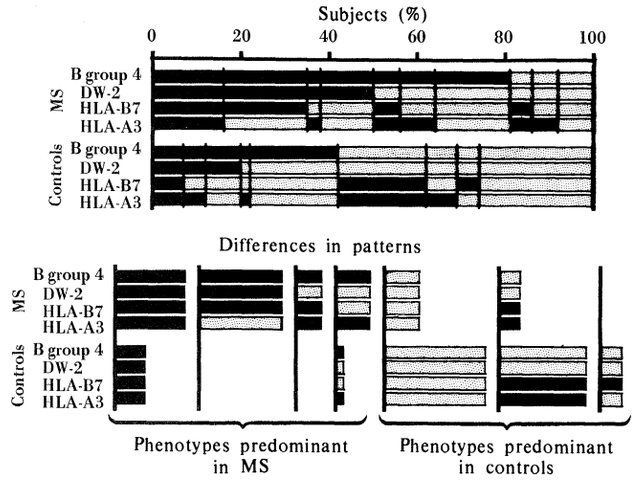




Fig. 1 (left). A series of 33 antiserums that define six B lymphocyte specificities were reacted with the B lymphocytes of 38 MS patients (shown by asterisks); the reactivity to controls is shown by circles. The vertical line showing the difference in reactivity of a given serum to the two populations is a thin line when the frequency of reactivity is greater for the control population than the MS patients. The thick vertical line shows the serums which had reacted to a higher frequency with MS patients. These serums fell with group 4. Fig. 2 (right). The relation of HLA-A3, HLA-B7, DW2, and group 4 is given here for the 38 MS patients and the 72 controls. The presence of a given antigen is denoted by the dark bar and absence by the shaded bar. For example, 16 percent of the MS patients have B group 4, DW2, HLA-B7, and HLA-A3. The bottom half of the figure shows the comparisons of a given set of patterns in the MS and control groups as derived from the upper half. Persons with all four specificities are much more frequent among MS patients, and persons who lack all four specificities are more frequent among controls.

tween the two groups. Serums are arranged in groups according to the classifications arrived at through testing of normal B lymphocytes (*6*). All five serums in group 4 have a high frequency of reactivity in MS patients. One serum reacted with 92 percent of the MS patients and 58 percent of the controls. The difference in reactivity was highly significant for all five serums with chi-square values of 14.9, 30.3, 8.1, 12.9, and 6.4, respectively.

The first two serums reacted with normal controls at a lower frequency than the last three (Fig. 1). Because the serums sometimes did not react concordantly, the cells were classified as having group 4 when three out of the five serums reacted with a cell. Weak reactions and false positive or negative reactions with any given serum will tend to be compensated by this procedure. When 18 other MS patients, typed subsequently, were added to the first series of 38 patients, the frequency of group 4 was 83.9 percent in 56 MS patients. Among the 72 controls, the frequency of group 4 was 32.5 percent. The difference was statistically significant ($P < .00005$, and after correcting for the six specificities tested, $P < .0003$).

Since these specificities appear to belong to a single genetic locus (*6*), an increased frequency of group 4 would be expected to result in a decreased frequency in the other allelic specificities. Such a decrease in frequencies of serums in groups 1, 2, 3, and 5 was found. This finding emphasizes the fact that the difference in reactivity of MS and normal B lymphocytes is not attributable to B lymphocytes being more sensitive to complement-dependent cytotoxicity than T lymphocytes. Serums classified in the other groups were more reactive with B lymphocytes from normal persons than from MS patients.

The combined presence of HLA-A3, HLA-B7, DW2, and B group 4 in the first 38 MS patients and controls is shown in Fig. 2. The patterns of reactivity are distinct in these two populations. All four specificities are more common among the MS patients as compared to the controls. The combination of B group 4, DW2, and HLA-B7 without HLA-B3 was not found among the controls, but was frequent in the MS patients. Significantly, DW2 was entirely included in group 4; that is, every individual who was DW2 also showed group 4 antigen. Therefore, the HLA-D locus is probably closely related to the locus that determines the group 4 antigen. In contrast, HLA-B7 is almost completely included in the group 4 antigen among MS patients, but not among the normal population.

That there are no persons with all four antigens in the normal group as compared to only a small number among the MS patients suggests that the MS susceptibility gene is more frequently linked with haplotypes containing one of the four MS-associated specificities. The data given in Fig. 2 reinforce and extend the concept that the MS susceptibility gene is present only on a limited number of HLA chromosomes (*8*). The HLA genetic markers that are "characteristic" or often in linkage disequilibrium with the MS susceptibility gene are HLA-A3, HLA-B7, HLA-DW2, and group 4. We now see that the entire set of these four specificities is probably in linkage disequilibrium with the MS susceptibility gene.

The exact haplotype composition cannot be determined without extensive family studies, although methods by which they can be determined from random populations of MS patients have been described (*8*). Results of family studies have shown that the incidence of HLA-A3, HLA-B7 haplotype is significantly higher in MS patients (9.2 percent) as compared to controls (6.3 percent) (*9*). From the strong association of HLA-B7 and DW2, it can be inferred that a linkage disequilibrium exists between these two specificities (*3*). The data given in Fig. 2 suggest that haplotypes carrying the MS susceptibility gene have the group 4 specificity, DW2, as well as often having the HLA-B7 and HLA-A3 specificities. From an independent study of 60 haplotypes in 30 families, a linkage between HLA-B7 and group 4 was established (*10*).

Such a high association of products from different loci could be attributed to the entire set of specificities being selected out in the evolutionary process. Alternatively, a fusion of two populations as a result of migration may have produced the linkage disequilibrium (*11*). The original MS susceptibility mutation may have occurred in one individual who was HLA-A3, HLA-B7, HLA-DW2, and group 4. Crossovers have led to the presence of the MS susceptibility gene on other haplotypes. As we noted earlier (*1*), the original population in which this mutation arose probably was in northern Europe, where the frequencies of HLA-A3 and HLA-B7 are high. From prelim-

inary findings in other populations, such as that in Japan, an independent mutation appears to be involved.

Undoubtedly, many more associations will be uncovered. Also, from this example, it appears that disease susceptibility is determined by a locus on the HLA chromosome, but that the locus is closer to the B lymphocyte antigen locus than to the HLA-A, -B, -C, or -D loci. This is inferred from the fact that association with MS is less with the A and B loci and somewhat less with the D locus. The finding that everyone who is DW2 is also group 4 (in this small series), suggests that the antigens could indeed be identical, but that the two different methods may produce different results. It is possible that HLA-D locus testing may not identify all the group 4 positive cells.

An association with DW3 has been found in celiac disease and dermatitis herpetiformes (*12*), which was higher than the earlier reported association with HLA-B8 (*13*). In another study two antiserums that react to a higher frequency with B lymphocytes of such patients were described (*14*). Whether the serums react with DW3 cells was not determined, although the possibility that they detect antigens similar to Ia antigens was postulated. The existence of an IR (immune response) gene for MS susceptibility and the detection of Ia antigens by antibodies to B lymphocytes may be analogous to the system described in mice (*15*).

Since not all MS patients in our study were of the group 4 type, it could be postulated that "pure" MS will prove to be of a specific type when the MS patients are finally accurately diagnosed. The serums defining group 4 may also be changed eventually since, as discussed earlier, the reactions are heterogeneous. It is perhaps more likely that because of crossing-over, the MS susceptibility gene is present on haplotypes other than that with group 4.

The above results indicate that the B group 4 could be utilized as a diagnostic aid, even though the rather high incidence of the antigen group in normal subjects may be a complicating factor. However, if some clinical signs of MS are viewed in combination with B lymphocyte typing, the possibility of accurately identifying MS patients may be magnified considerably.

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# Endolithic Blue-Green Algae in the Dry Valleys: Primary Producers in the Antarctic Desert Ecosystem

Abstract. *Endolithic unicellular blue-green algae occur under the surface of orthoquartzite rocks in the dry valleys of southern Victoria Land, Antarctica. This report of primary producers in the Antarctic desert ecosystem suggests that, in future efforts to detect life in extraterrestrial (for example, martian) environments, scientists should consider the possible existence of endolithic life forms.*

The ice-free cold desert of the dry valley region in southern Victoria Land, Antarctica (77°30′S, 161°00′E) can be regarded as the most extreme and inhospitable environment on Earth. This area attracted the interest of microbiologists not only because the combination of extreme drought and cold poses utmost demands on the adaptive capacity of microorganisms (*1*), but also because the dry valleys are regarded as the closest terrestrial analog to martian or other extraterrestrial planetary environments (*2*, *3*).

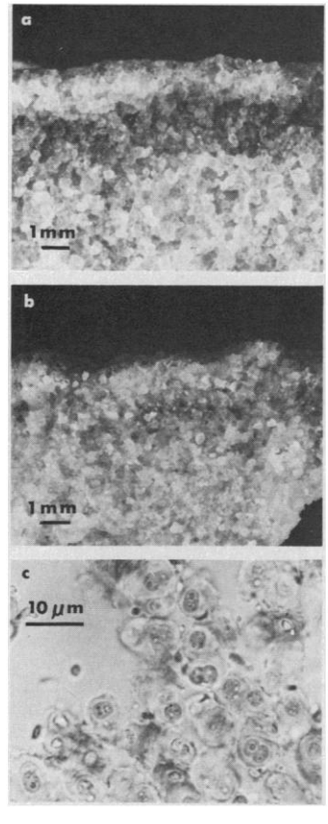


Fig. 1. (a) Vertically fractured orthoquartzite rock from the Mount Baldr–Mount Thor area, Wright Valley, Asgard Range, southern Victoria Land, showing a dark green zone of endolithic algae under the surface. (b) Similar endolithic algal zone in Nubian sandstone, from Wadi Mangan, north of Timna, Negev Desert. (c) Unicellular blue-green algae (*Gloeocapsa* sp.) from rock shown in (a).

Horowitz *et al.* (*2*) summarized the results of soil microbiological research in the dry valleys. They concluded that either (i) dry valley soils are abiotic or (ii) when microorganisms do appear in cultures, they are not indigenous. The authors suggested that the microbial population of dry valley soils represents a steady state in which influx from the atmosphere (carried by wind from more favorable environments) is balanced by mortality on the ground. These findings were challenged by Vishniac and Mainzer (*3*, *4*), who found an indigenous soil microflora by microbiological methods applied in situ. Yeasts recently isolated