lymphocytes are producing antitetanus antibody. With each succeeding generation in vitro, the antibody-producing cells, apparently not those best adapted to the tissue culture conditions, constitute less and less of the total cell population. This is consistent with the limiting dilution data. It is also consistent with the multiclonal infective potential of Epstein-Barr virus (*16*).

We believe that we have established continuous lymphoid lines producing human antibody to tetanus toxoid in quantities that might make stable clones of these lines a source of specific human antibody. Since we have observed specific antibody in cell culture media obtained from microtiter wells initiated with as few as ten cells and since the cloning efficiency on human foreskin fibroblasts is relatively high, it seems likely that homogeneous, stable antibody-producing clones will shortly be available. This approach may lead to a general method for production of specific monoclonal human antibodies in vitro.

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B Lymphocyte Antigens in Sicca Syndrome

Abstract. *All individuals tested in this study with sicca syndrome, a human autoimmune disease, bear two immunologically distinct and genetically unrelated B lymphocyte antigens that appear similar to the immune response associated (Ia) antigens of the mouse. The genes coding for these two antigens are present in only 37 and 24 percent of normal controls. In animal models Ia antigen genes are closely linked to immune response genes. Our findings suggest that two such genes may be required for the development of sicca syndrome.*

Loci of the major histocompatability complex (MHC) of man, located on chromosome 6, code for a series of human lymphocyte antigens (HLA). The seriologically defined antigens, HLA-A and -B, are widely represented on many body tissues in addition to lymphocytes. The lymphocyte-defined HLA-D region antigens, typed by the mixed lymphocyte reaction, are distinct from the HLA-A and -B antigens. Additional genes of the MHC, some of which are closely linked to the HLA-D region, code for antigens restricted to lymphocytes bearing surface immunoglobulin and Fc-receptor (B cells). These structures appear analogous to the immune response associated (Ia) antigens of mice. In that species, the genes coding for Ia antigens map in the same region as immune response (Ir) genes. Antiserums have been developed which recognize human B cell Ia-like molecules. These allow more precise definition of these potentially immunoregulatory cell surface antigens (*1*,*2*).

The autoimmune disease, sicca syndrome, also known as Sjögren's syndrome, consists of dry mouth and dry eyes caused by immunologic destruction of the salivary and lacrimal glands. Patients with this disorder have rheumatoid factor and other circulating autoantibodies which suggests that a disordered immune response may play a role in the pathogenesis of this disease (*3*).

Sicca syndrome is associated with a specific histocompatability antigen, HLA-B8, which is expressed in 50 to 55 percent of patients but only 20 percent of controls (*4*). Two subsequent studies revealed stronger (69 percent), but not absolute, association of sicca syndrome with the HLA-DW3 allele, which is in linkage disequilibrium with HLA-B8 (*5*), and suggested that sicca syndrome is primarily associated with this lymphocyte-defined antigen rather than HLA-B8.

The availability of antiserums to Ia-like antigens makes it possible to ascertain which specificities are associated with sicca syndrome. We determined the B lymphocyte antigens in 24 patients (21 females and 3 males, ages 26 to 73) and 184 normal controls (96 females and 88 males, ages 18 to 51) (*6*). Three of the patients have rheumatoid arthritis and three have systemic lupus erythematosus in addition to sicca syndrome.

Immunoglobulin-bearing lymphocytes were prepared as described (*1*, *7*). The 60 antiserums used were obtained from multiparous women and absorbed with pooled platelets to remove HLA-A, -B,

Table 1. B lymphocyte antiserums which distinguish patients with sicca syndrome from normal controls.

Antiserum	Sicca syndrome		Normal controls		χ^2	P*
	Positive	Negative	Positive	Negative		
Ia-172	24	0	68	116	31.7	<.001
Ia-AGS	24	0	44	140	52.5	<.001
Ia-35	16	8	31	153	27.3	<.001
Ia-350	15	9	39	145	16.8	.002
Ia-590	14	10	37	147	14.8	.007
Ia-715	13	11	26	158	19.8	<.001

*Corrected for the number of antiserums tested.

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and -C antibodies. The purified B lymphocytes were tested by a complement-dependent cytotoxicity assay (*1, 7*). Peripheral blood lymphocytes were typed for the HLA-A and -B antigens by the microdroplet lymphocyte cytotoxicity assay (*8*).

The results obtained with antiserums that detected significant differences between the sicca syndrome and control groups are shown in Table 1. Two antiserums, 172 and AGS, reacted with the B lymphocytes from all the sicca syndrome patients, compared to 37 and 24 percent of the control population, respectively. Four additional antiserums (35, 350, 590, and 715) reacted more frequently (67, 63, 58, and 54 percent) with the patients' B cells than with those of the controls (17, 21, 24, and 14 percent).

In order to determine whether antiserums 172 and AGS recognized the same or different antigens, their reactions with the lymphocytes from the 184 normal individuals were compared by the χ^2 test. All the correlations significant at $P < .05$ are shown in Table 2. For example, Ia-172 correlated with both Ia-35 and Ia-350 but not with Ia-AGS or Ia-715; Ia-AGS correlated only with Ia-715. While there is some indication that serums 172 and AGS recognize more than one antigen, it is not yet known whether these correlations represent immunologic cross-reactivity or linkage disequilibrium. Table 2 also shows that Ia-35 is in linkage disequilibrium with HLA-B8. A similar analysis for the patients is presented in Table 3. Because of their 100 percent coincidence in sicca syndrome, 172 and AGS are highly related to all the specificities examined and are not included in Table 3. In the patient group, Ia specificities 35, 350, and 590 are associated with each other but not with 715. Again, this could be due to either cross-reactivity or linkage disequilibrium.

Of the 24 sicca syndrome patients, 19 have also been typed for HLA-DW3 (*5*). The coefficient of contingency between DW3 and Ia-590 is .53, $P = .007$, and between DW3 and Ia-35 is .39, $P = .07$. DW3 did not correlate with Ia-350 or Ia-715. These results demonstrate high concordance between HLA-D type and certain antiserums to Ia but not others.

Thus B cells of all our patients with sicca syndrome bear two Ia specificities, 172 and AGS, that are not associated in the normal population. It should be noted that these specificities are defined by the reactivities of a single serum. Further investigation is needed to improve the serological definition of Ia-like antigens and their associations with disease states. In contrast, the single HLA-DW3 specificity is found in 69 percent of sicca patients (*5*). As would be expected, the patient group also shows a higher incidence of Ia specificities that are associated with these two in normal individuals: Ia-35 and -350, which are associated with Ia-172 and Ia-715, which is associated with AGS. The similar associations of Ia-172 and -AGS with other specificities in sicca patients and controls makes it unlikely that both antiserums recognize a single Ia antigen that is unique to the patient group.

The presence of both Ia-172 and -AGS is not itself sufficient for the development of sicca syndrome since approximately 9 percent of the population would be expected to carry both specificities. The nature of the other factor or factors involved in the pathogenesis of the disorder is unknown at present.

An association has also been shown between multiple sclerosis and a B lymphocyte antigen in a study of 25 patients (*9*). Since multiple sclerosis is associated with HLA-B and -D specificities (*9*) different from those of sicca syndrome, it is likely that the B cell antigens are also distinct.

The susceptibility to experimental autoimmune thyroiditis in mice, which is controlled by the MHC, has been shown to be regulated by either the K region or the I-A subregion of the Ia portion of the MHC. This result is compatible with susceptibility to an experimental autoimmune disease being controlled by the Ir region (*10*).

Assuming that Ia-like B lymphocyte antigens in humans are coded by an Ir region, our results suggest that two immune response genes may be involved in the pathogenesis of sicca syndrome. The absolute association of the Ia-172 and AGS antigens with sicca syndrome should provide an additional aid in the diagnosis of this disorder. Whether the same Ia specificities are also found in the other autoimmune diseases associated with HLA-B8 remains to be determined.

Table 2. Correlation between various HLA and Ia specificities in normal controls. Only the significant correlations are shown.

	Ia-35	Ia-350	Ia-715	Ia-AGS
Ia-172	.37* <.001†	.59 <.001†		
Ia-AGS			.36 .002†	
HLA-B8	.45‡ <.001†			

*Coefficient of contingency. †P value. ‡The calculated gene frequencies of B8 and Ia-35 are .094 and .079, respectively. The expected frequency of the B8 and Ia-35 haplotype would be the product of the two gene frequencies, .007. The actual B8 and Ia-35 haplotype frequency is .040, five times the expected, indicating linkage disequilibrium between B8 and Ia-35.

Table 3. Correlation between various Ia specificities in patients with sicca syndrome.

	Ia-35	Ia-350
Ia-590	.48* .007†	.39 .04†
Ia-350	.43 .02†	

*Coefficient of contingency. †P value.

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