duce similar fivefold increases of $[Ca^{2+}]_i$ in pancreatic acinar cells and gastric smooth muscle cells of the guinea pig, and preloading with quin2 inhibits maximal response in both cell types to the same extent (29 to 32 percent) (*8*). However, agonist-induced secretory and metabolic responses in pancreatic acinar and liver cells have yet to be measured with the speed necessary to establish the kinetic sequence or the stoichiometric coupling of $[Ca^{2+}]_i$ and cell response. These relations are more easily demonstrated in isolated muscle cells as shown in Fig. 1 for the kinetic sequence and in Fig. 2 for the stoichiometric coupling of peak $[Ca^{2+}]_i$, peak net Ca^{2+} efflux, and peak contraction.

Studies with saponin-permeabilized gastric muscle cells suggest that release of intracellular Ca^{2+} is mediated by inositol 1,4,5-trisphosphate ($InsP_3$) (*12, 13*). The water-soluble derivative of phosphatidylinositol 4,5-bisphosphate releases Ca^{2+} from the same pool as that shown to be the source of the intracellular Ca^{2+} released by CCK-8 and acetylcholine. The maximal increase in $[Ca^{2+}]_i$, net Ca^{2+} efflux, and contraction elicited by $InsP_3$ are similar to those elicited by CCK-8 and acetylcholine, consistent with a role for $InsP_3$ as the membrane-derived messenger for mobilization of intracellular Ca^{2+}.

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Gene Interaction at HLA-DQ Enhances Autoantibody Production in Primary Sjögren's Syndrome

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Primary Sjögren's syndrome is an autoimmune disorder characterized by dryness of the mouth and eyes. The human leukocyte antigen (HLA) locus DQ is related to the primary Sjögren's syndrome autoantibodies that bind the RNA proteins Ro/SSA and La/SSB. Both DQ1 and DQ2 alleles are associated with high concentrations of these autoantibodies. An analysis of all possible combinations at DQ has shown that the entire effect was due to heterozygotes expressing the DQ1 and DQ2 alleles. These data suggest that gene interaction between DQ1 and DQ2 (or alleles at associated loci), possibly from gene complementation of *trans*-associated surface molecules, influences the autoimmune response in primary Sjögren's syndrome.

THE MAJOR HISTOCOMPATIBILITY complex, which is known in humans as the HLA system, has an important role in controlling immunologic defense mechanisms. Recent advances in the model for the genetic organization of the HLA system have resulted in a redefinition of the class II loci as HLA-DP (previously SB), HLA-DQ (previously DS, DC, and MB), and HLA-DR (unchanged) (*1*). The cell surface molecules encoded by class II genes are composed of pairs of transmembrane peptides termed α and β. The HLA-DR polymorphisms defined between individuals are a result of allelic variations in β chain structure. No polymorphism has been recognized in the HLA-DR α chain. Both the α and the β HLA-DQ chains are polymorphic, and *trans*-associations as well as *cis*-associations of these peptides have been described (*2*).

Diseases associated with an HLA specificity in which an antigen is known and in which the response to the antigen clearly causes the disease are rare. Myasthenia gravis is perhaps the best example. This disorder is associated with the B8,DR3 haplotype in caucasoids, and all available evidence supports the hypothesis that it is caused by autoantibody to the acetylcholine receptor (*3*).

The primary form of Sjögren's syndrome or the sicca complex is another autoimmune disease associated with B8,DR3 (*4*). It is characterized by lymphocytic infiltration of exocrine glands, particularly the salivary and lacrimal. Patients with this disorder complain of dry mouth and eyes. Extraglandular manifestations including vasculitis and purpura as well as central nervous system, muscle, hematologic, kidney, and lung involvement may be present. When this disorder occurs in the absence of another diagnosis, it is referred to as primary Sjögren's syndrome. The sicca complex is commonly found in patients with other rheumatic diseases including rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis, and polymyositis. These patients are referred to as having secondary Sjögren's syndrome. Sicca complex is very common; estimates of prevalence in populations over 55 years old exceed 1 percent (*5*).

Antibodies to Ro/SSA and La/SSB are found in nearly all Sjögren's syndrome patients, and many of the extraglandular features are associated with high concentrations of these autoantibodies (*6*). The Ro/SSA and La/SSB antigens are RNA-protein complexes, and the RNA's are RNA polymerase III products. La/SSB binds to terminal uridine-rich regions of RNA polymerase III transcripts (*7*).

An evaluation of these autoantibodies and the DQ alleles in Sjögren's syndrome was undertaken when it was discovered that both DQ1 and DQ2 might be associated with elevated binding of antibodies to Ro/SSA and La/SSB ($0.08 > P > 0.03$) (*8*). Further analysis of the HLA-DQ relations to Ro/SSA and La/SSB antibodies showed that the entire effect of DQ was found in the patients with primary Sjögren's syndrome (Table 1). The associations with both DQ1 and DQ2 raised the possibility that particular combinations of HLA-DQ alleles could account for this effect. Indeed, when all possible combinations of the HLA-DQ alleles were evaluated for Ro/SSA and La/SSB antibodies, only patients heterozy-

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gous for the DQ1 and DQ2 haplotypes showed a highly positive association (Table 2). No relations were found when the patients with secondary Sjögren's syndrome were similarly analyzed, neither in the aggregate nor when the patients with each diagnosis were analyzed as subgroups: rheumatoid arthritis, $n = 15$; systemic lupus erythematosus, $n = 16$; other, $n = 5$.

Potentially important associations were present at other allelic combinations, particularly DQ1/DQ– and DQ2/DQ– (where DQ– indicates homozygosity at DQ and the possibility of a second allele that has not been detected). By removing the DQ1/DQ2 patients and repeating the analysis, these effects became less statistically significant. Indeed, after removing the DQ1/DQ2 patients, the patients with DQ1/DQ– or DQ2/DQ– still had lower mean concentrations of Ro/SSA and La/SSB antibodies than the remaining patients did. This result confirms that the differences found when each allele was considered alone (Table 1) were almost entirely due to the DQ1/DQ2 heterozygous individuals (in Table 2). Removing the DQ1/DQ2 heterozygotes, however, did reveal a borderline effect in the DQ2/DQ3 heterozygous patients for La/SSB antibodies ($P < 0.06$) (*9*).

Antibodies to Ro/SSA and La/SSB are associated with many other manifestations of Sjögren's syndrome (*6*). Table 3 shows that particular findings are also associated with DQ1/DQ2, especially those related to the globulin level. In this data set, DQ1/DQ2 was not related to antibodies to nRNP(Sm), parotid enlargement, lymphadenopathy, sex, race, lymphopenia, thrombocytopenia, or age.

The impressive difference in immunoglobulin G (IgG) between the DQ1/DQ2-positive patients and the remainder is reflected in each of the autoantibodies important in primary Sjögren's syndrome: Ro/SSA and La/SSB antibodies, rheumatoid factor, and antinuclear antibody. The effect related to the DQ1/DQ2 interaction may therefore be related to the production of multiple autoantibodies and may not be constrained to the production of a single specificity. On the other hand, the realization that autoantibodies may have multiple specificities raises the possibility that to some extent these assays measure the concentration of the same autoantibody (*10*). Indeed, neither Ro/SSA nor La/SSB antibodies make a contribution to the abnormal serologic findings in the DQ1/DQ2 heterozygotes which is independent of the other (*11*).

Though the proportion of primary Sjögren's syndrome patients in this study with the DQ1/DQ2 allelic combination (30 percent) is greater than in 1258 normal controls (18 percent), the difference is not statistically significant ($\chi^2 = 2.74$, $P \equiv 0.1$; relative risk 1.70) (*12*). From these data, we cannot conclude that DQ1/DQ2 increases the risk of developing primary Sjögren's syndrome, but higher concentration of selected autoantibodies are likely in these individuals. DR3, on the other hand, is present in 26 of the 48 patients, and it increases the risk of Sjögren's syndrome ($\chi^2 = 9.7$,

Table 1. Antibodies to Ro/SSA and La/SSB binding units in relation to HLA-DQ in primary Sjögren's syndrome. Informed consent for participation was obtained from patients who satisfied diagnostic criteria (*4, 6*). Alleles at the HLA-DQ locus were determined by microcytotoxicity assay (*4*). Antibodies to Ro/SSA and La/SSB were determined by solid-phase enzyme-linked immunosorbent assays with highly purified autoantigen preparations (*7, 15*). One unit of autoantibody is equivalent to the limit of detection in the assay, which is a 10^{-7} dilution of an arbitrarily selected standard serum. The antilog of the $\log_{10}$ mean multiplied or divided by the standard error of the $\log_{10}$ least-squares mean is presented. Groups were compared by t tests with the least-squares means. Normal individuals with no evidence for autoantibody had less than 10,000 units of antibody to Ro/SSA and less than 3000 units of antibody to La/SSB binding activity. The n indicates the number of patients who had (+) or who did not have (−) the designated DQ allele.

Allele	n	Antibody to Ro/SSA	P	Antibody to La/SSB	P
DQ1	+32	450,000 ×÷ 0.260	0.0085	30,500 ×÷ 0.238	0.0020
	−16	124,000 ×÷ 0.379		10,600 ×÷ 0.348	
DQ2	+27	549,000 ×÷ 0.242	0.0048	45,300 ×÷ 0.222	0.0006
	−21	130,000 ×÷ 0.328		8,240 ×÷ 0.301	
DQ3	+19	205,000 ×÷ 0.286	0.25	19,900 ×÷ 0.262	0.058
	−29	369,000 ×÷ 0.309		22,600 ×÷ 0.283	

Table 2. Analysis of HLA-DQ alleles in primary Sjögren's syndrome for antibodies to Ro/SSA and La/SSB. All possible allelic combinations at HLA-DQ are presented as detected by microcytotoxicity assay. DQ– indicates an individual who is homozygous for the only detected allele at DQ or who has a second allele which has not been detected. The DQ1/DQ2 heterozygotes had an average of approximately 0.5 mg/ml of antibody to Ro/SSA (*16*).

Allelic combinations	n	Antibody to Ro/SSA	P	Antibody to La/SSB	P
DQ1/DQ–	+12	90,300 ×÷ 0.388	0.17	5,270 ×÷ 0.374	0.070
	−36	372,000 ×÷ 0.215		32,700 ×÷ 0.207	
DQ2/DQ–	+4	22,000 ×÷ 0.635	0.081	1,510 ×÷ 0.618	0.058
	−44	337,000 ×÷ 0.194		27,300 ×÷ 0.188	
DQ1/DQ2	+13	2,120,000 ×÷ 0.329	0.0024	174,000 ×÷ 0.319	0.0016
	−35	122,000 ×÷ 0.203		9,550 ×÷ 0.197	
DQ3/DQ–	+2	23,500 ×÷ 0.916	0.25	2,615 ×÷ 0.899	0.31
	−46	297,000 ×÷ 0.193		23,400 ×÷ 0.189	
DQ1/DQ3	+7	182,000 ×÷ 0.496	0.72	19,100 ×÷ 0.486	0.92
	−41	286,000 ×÷ 0.208		21,700 ×÷ 0.203	
DQ2/DQ3	+10	343,000 ×÷ 0.415	0.77	30,700 ×÷ 0.406	0.66
	−38	250,000 ×÷ 0.216		19,300 ×÷ 0.211	

Table 3. Relation of the DQ1/DQ2 complementation group to findings in Sjögren's syndrome. Continuous data are compared by t tests; means ± or ×÷ SEM are presented. The antilog_2 of the $\log_2$ mean of the antinuclear antibody (ANA) and rheumatoid factor (RF) titers are compared. Chi square with the Yates correction was used to test the categorical data of leukopenia (two different determinations of <4000 white blood cells per cubic millimeter).

DQ1/DQ2	Globulin (mg/ml)	IgG (mg/ml)	IgA (mg/ml)	IgM (mg/ml)	ANA (titer)	RF (titer)	Leukopenia (number positive)
Positive ($n = 13$)	43.5 ± 3.8	20.5 ± 2.5	3.2 ± 0.5	2.1 ± 0.4	1:245 ×÷ 0.73	1:245 ×÷ 0.75	4 (31%)
Negative ($n = 35$)	29.8 ± 1.3	11.1 ± 1.2	2.3 ± 0.2	1.8 ± 0.4	1:83 ×÷ 0.42	1:53 ×÷ 0.42	3 (9%)
P	0.004	0.0005	0.07	0.7	0.06	0.01	0.06

$P < 0.01$; relative risk 2.55) when compared with a normal control population (398 of 1258).

A number of molecular mechanisms are possible. Gene interaction at HLA-DQ or with an associated locus may lead to enhanced autoimmune responsiveness. DR3 is in linkage disequilibrium with DQ2. Since 12 of the 13 patients who were positive for DQ1/DQ2 were also positive for DR3 (and since the one exception had a lower concentration of autoantibody), perhaps the important interaction is between DR3 and DQ1. Consistent with this hypothesis is the observation that DR3 is significantly associated ($\chi^2 = 12.3$, $P < 0.001$) with the DQ1/DQ2 heterozygous state in primary Sjögren's syndrome (12 of 13); the comparable proportion in a normal control population of DQ1/DQ2 heterozygotes was 92 of 223. Though formally a possibility, there are no data to support the notion that hybrid molecules can be formed as a result of *cis*- or *trans*-association between the products of HLA-DR and HLA-DQ. On the other hand, gene complementation occurs at HLA-DQ, resulting in two hybrid molecules that arise from *trans*-association of the β chain from one chromosome and the α chain from the other. These molecules carry epitopes that may not be found in either parent (*2*).

The relationships of DR3 to DQ1 as opposed to DQ2 are very different in primary Sjögren's syndrome. Of the 35 patients remaining after the DQ1/DQ2 heterozygotes are removed, 14 expressed the DR3 antigen. In two, DR3 occurred with DQ1 and without DQ2. The 12 DR3-positive, DQ2-positive, and DQ1-negative patients remaining represent nearly all of the 14 patients who express DQ2/DQ– or DQ2/DQ3 and who have much lower concentrations of autoantibodies than the DQ1/DQ2 heterozygotes (Table 2). The concurrence of DR3 and DQ2 is remarkable in these patients, with 24 patients having both alleles and only five having one allele without the other. Even after removing the DQ1/DQ2 heterozygotes from consideration, the presence of DR3 (14 of 35) increased the likelihood of having primary Sjögren's syndrome ($\chi^2 = 7.19$, $P < 0.01$; relative risk 2.68) compared with a control group from which the DQ1/DQ2 heterozygotes had also been removed (206 of 1035). These data suggest a model in which DR3 or a particular subset of DQ2 molecules closely associated with DR3 increase the risk of developing primary Sjögren's syndrome, while the interaction between DQ1 and DR3 or this particular DQ2 increases the production of autoantibodies related to Sjögren's syndrome.

That such a powerful effect (Table 2) results from gene interaction at or near HLA-DQ suggests that functions of the HLA-DQ gene products are important in the perpetuation, if not also the initiation, of this autoimmune response and related disease processes. Many immune functions, including antigen presentation and cellular cytotoxicity, among other immune cellular interactions, are restricted by major histocompatibility complex alleles. That is, the interacting cells bear similar, if not identical, alleles at the locus of the participating major histocompatibility complex. Hence, our data suggest that these immune events modulate the autoimmune process of primary Sjögren's syndrome.

In the mouse, the genes determining whether an immune response occurs to an immunogen has been mapped to the I region of the major histocompatibility complex. Of the known mouse major histocompatibility complex loci, the human DQ shares greatest sequence homology with I-A, whereas human DR is the homolog of mouse I-E (*13*). The finding of multiple autoantibodies in the DQ1/DQ2 heterozygous patients does not necessarily imply a nonspecific effect. The substances recognized as a consequence of such a gene interaction may define the range of possible autoimmune responses and determine the richness and variety of the collection of resulting autoantibodies.

The modulation of disease by genetic factors is well known. Sjögren's syndrome is most closely associated with DRw52 (previously known as MT2), which is a supertypic specificity encoded by a $DR\beta_2$ gene in linkage disequilibrium with DR3, DR5, DRw6, and DRw8 molecules (*4*). Precipitating Ro/SSA and La/SSB antibodies, on the other hand, have been associated with DR3 with a contribution from DR2 found in some studies (*4*). The linkage disequilibrium of DR3 with DQ2 and DR2 with DQ1 may explain these findings. Regardless of the detailed molecular mechanism, Sjögren's syndrome patients with the DQ1/DQ2 alleles have a much more severe autoimmune disease than do patients with any other alleleic combination at HLA-DQ.

In other diseases, multiple HLA alleles are associated with the presence of the disease. Both DR2 and DR3 have been associated with Ro/SSA precipitins in systemic lupus erythematosus (*4*). Individuals who have DR3 and DR4 are more likely to develop type I diabetes or herpes gestationis (*14*). Whether these data also reflect the immunoregulation induced by heterozygous hybrid molecules at HLA-DQ rather than other types of gene interaction at or near HLA-DR or HLA-DQ is not known.

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9. The DQ2/DQ3 heterozygotes ($n = 10$) had a concentration of La/SSB antibodies of 30,700 ± 0.306 units and the nonheterozygotes ($n = 25$) had 5880 ± 0.198 units. The Ro/SSA antibody concentration in this group was higher in DQ2/DQ3 heterozygotes 343,000 ± 0.341 units than in nonheterozygotes 78,300 ± 0.220 units, but did not achieve statistical significance ($P = 0.12$).
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11. Logistic regression analysis was performed with the DQ1/DQ2 heterozygous state of primary Sjögren's syndrome as the dependent variable and antibodies to La/SSB and Ro/SSA as independent variables. In the multiple model $\beta = 0.67 \pm 0.60$ (SEM) and $\beta = 0.11 \pm 0.58$ while $P = 0.26$ and $P = 0.85$ for the independent contribution of antibodies to La/SSB and Ro/SSA, respectively. Computations were made according to SAS Institute, Inc., *SUGI Supplemental Library User's Guide* (SAS Institute, Cary, NC, 1983), p. 181.
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