Differential Distribution of HLA-DQβ/DRβ Epitopes in the Two Forms of Guillain-Barré Syndrome, Acute Motor Axonal Neuropathy and Acute Inflammatory Demyelinating Polyneuropathy (AIDP): Identification of DQ β Epitopes Associated with Susceptibility to and Protection from AIDP

Eleni E. Magira, Miltiadis Papaioakim, Irving Nachamkin, Arthur K. Asbury, Chun Y. Li, Tony W. Ho, John W. Griffin, Guy M. McKhann and Dimitri S. Monos

J Immunol 2003; 170:3074-3080; ;
doi: 10.4049/jimmunol.170.6.3074
http://www.jimmunol.org/content/170/6/3074

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Differential Distribution of HLA-DQβ/DRβ Epitopes in the Two Forms of Guillain-Barré Syndrome, Acute Motor Axonal Neuropathy and Acute Inflammatory Demyelinating Polyneuropathy (AIDP): Identification of DQβ Epitopes Associated with Susceptibility to and Protection from AIDP[1]

Eleni E. Magira,* Miltiadis Papaioakim,† Irving Nachamkin,§ Arthur K. Asbury,¶ Chun Y. Li,‖ Tony W. Ho,# John W. Griffin,# Guy M. McKhann,# and Dimitri S. Monos[2]*‡**

Guillain-Barré syndrome (GBS), an acute, immune-mediated paralytic disorder affecting the peripheral nervous system, is the most common cause of acute flaccid paralysis in the postpolio era. GBS is classified into several subtypes based on clinical and pathologic criteria, with acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN) being the most common forms observed. To better understand the pathogenesis of GBS and host susceptibility to developing the disease, the distribution of HLA class II Ags along with the seroreactivity to *Campylobacter jejuni* were investigated in a population of GBS patients from northern China. Using DNA-based typing methods, 47 patients with AMAN, 25 patients with AIDP, and 97 healthy controls were studied for the distribution of class II alleles. We found that the DQβ RLD^{55-57}/ED^{70-71} and DRβ $E^{9}V^{11}H^{13}$ epitopes were associated with susceptibility to AIDP ($p = 0.009$ and $p = 0.004$, respectively), and the DQβ RPD^{55-57} epitope was associated with protection ($p = 0.05$) from AIDP. These DQβ/DRβ positional residues are a part of pockets 4 (DQβ 70, 71, DRβ13), 6 (DRβ11), and 9 (DQβ 56, 57, DRβ9); have been demonstrated to be important in peptide binding and T cell recognition; and are associated with other diseases that have a pathoimmunological basis. Class II HLA associations were not identified with AMAN, suggesting a different immunological mechanism of disease induction in the two forms of GBS. These findings provide immunogenetic evidence for differentiating the two disease entities (AMAN and AIDP) and focuses our attention on particular DRβ/DQβ residues that may be instrumental in understanding the pathophysiology of AIDP. ***The Journal of Immunology*****, 2003, 170: 3074–3080.**

Guillain-Barré syndrome (GBS)[3] is an acute, immune-mediated attack on the peripheral nervous system resulting in a progressive sensory loss and/or motor weakness. GBS has an incidence rate of 0.4–4.0 per 100,000 people/year and represents the most common cause of acute neuromuscular paralysis since the decline in the number of polio cases (1, 2). GBS is a heterogeneous disorder based on clinical, electrophysiologic, and pathologic characteristics and includes several major subtypes: acute inflammatory demyelinating polyneuropathy (AIDP) (3–5), acute motor axonal neuropathy (AMAN) (6, 7), and Fisher syndrome (8).

In AIDP, macrophage-mediated demyelination and lymphocytic infiltrates around the nerve are observed, suggesting that AIDP is a T cell-mediated disorder (4, 9). This is supported by the lymphocytic inflammation found in many cases and the similarity to the pathology found in animals with experimental allergic neuritis (10–12). There is also evidence for the role of complement-mediated Ab attack on the nerve in the pathogenesis of AIDP (13).

In AMAN, electrophysiologic features suggest purely motor axon involvement without demyelination. Lymphocytic infiltration, as observed in AIDP, is nearly absent (6, 9, 14, 15). Evidence of complement-mediated Ab damage at the node of Ranvier, followed by attack of axons by macrophages, suggest an entirely different mechanism of nerve damage from AIDP (16). The classification between the primary axonal and primary demyelinating forms of the disorder is important because it provides the basis for clarifying the pathophysiology of the processes that lead to disease development. Furthermore, classification facilitates therapeutic approaches, as the efficacy of current treatments has been demonstrated mostly in patients with the primary demyelinating variant and not the axonal form (17).

GBS frequently follows a variety of presumed viral and bacterial infections, and *Campylobacter* gastroenteritis has been shown to be the single most identifiable agent associated with GBS (18). *Campylobacter* is one of the most common causes of bacterial

*Department of Pediatrics, University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, PA 19104; †Department of Medicine and ‡Laboratory of Human Biology and Genetics, Democritus University of Thrace School of Medicine, Alexandroupolis, Greece; Departments of §Pathology and Laboratory Medicine and ¶Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; ‖Second Affiliated Teaching Hospital, Hebei Medical School Shijiazhuamg, People's Republic of China; #Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287; and **Zanvyl Krieger Mind/Brain Institute, Baltimore, MD 21218

Received for publication May 28, 2002. Accepted for publication December 23, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grant NS31528 (to I.N., A.K.A., J.W.G., G.M.M., and D.M.).

[2] Address correspondence and reprint requests to Dr. Dimitri Monos, Children's Hospital of Philadelphia, 1208B Abramson Research Building, 3516 Civic Center Boulevard, Philadelphia, PA 19104. E-mail address: monosd@email.chop.edu

[3] Abbreviations used in this paper: GBS, Guillain-Barré syndrome; AMAN, acute motor axonal neuropathy; AIDP, acute inflammatory demyelinating polyneuropathy; BH, beryllium hypersensitivity; SSP, sequence-specific primer; SSOP, sequence-specific oligonucleotide probe; rr, relative risk.

0022-1767/03/$02.00

gastroenteritis in the U.S. and worldwide (19, 20). Patients are frequently exposed to *Campylobacter*; however, only 1 in 1000 develops GBS following infection (21). This strongly suggests that host susceptibility plays an important role in the development of GBS following infection (22).

There have been several studies of HLA associations with GBS, with a variety of associations identified; however, patients have not been characterized according to disease subtype, and this may explain the lack of consistent findings (23–28). We have had the unique opportunity to study GBS in a population of individuals from northern China who developed both AIDP and AMAN forms of GBS (6, 9, 29). To identify host susceptibility factors that may be involved in the development of AIDP or AMAN and to help resolve whether AIDP and AMAN are indeed different pathologic processes, HLA class II analysis (DRB1-, DQB1-, and DPB1 DNA-based typing) was performed on this population.

Materials and Methods

Population study

Subjects with AIDP and AMAN and controls were individuals from northern China who were referred to the Second Teaching Hospital in Shijiazhuang and Beijing Children's Hospital, People's Republic of China. All patients satisfied accepted diagnostic criteria (30), and they are classified into two groups: AMAN form of GBS ($n = 47$) and AIDP form of GBS ($n = 25$). Ninety-seven control subjects from the same area were also included in the study; they had no history of GBS, no family antecedent with GBS, nor any peripheral neuropathy and were randomly chosen.

DNA isolation

Whole blood from all these subjects was placed on filter paper (no. 1; Whatman, Clifton, NJ) and was carried back from China in individual plastic bags. Genomic DNA was extracted with Chelex-Resin 100 (Bio-Rad, Richmond, CA) according to the method of Walshet et al. (31).

DNA-based typing

Locus- and allele-specific amplification of genomic DNA were performed for DRB1-, DQB1-, and DPB1-associated alleles (32). A 270-bp amplified DNA product was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and was visualized under UV light. Hybridization was performed as described previously using a panel of sequence-specific oligonucleotide probes (SSOP) (33–35). The sequence-specific primer (SSP)-PCR using ARMS (European patent 0332435, U.S. patent 5595890, under license from AstraZeneca, Wilmington, DE) technology was used for high resolution of HLA-DRB1, HLA-DQB1, and HLA-DPB1 typing. Depending on the low resolution results of DRB1, DQB1 typing, appropriate kits (Olerup SSP; Genovision (West Chester, PA) and Biosynthesis (Lewisville, TX)) were chosen for subtyping of DRB1 and DQB1 loci (high resolution typing). When a specific allele could not be determined by SSOP or SSP-PCR, it was sequenced. The DNA was amplified using SSOP primers (32), and the sequences were analyzed in both forward and backward directions.

Table I. *Distribution of HLA-DRB1 alleles among control, AMAN and AIDP cases, and* C. jejuni *seropositive and seronegative patients*

	Control		AMAN				AIDP			
Alleles	*n* (97)	%	*n* (47)	%	Sero +	Sero −	*n* (25)	%	Sero +	Sero −
DRB1*0101	5	5.1	2	4.2	1	1	2	8.0	0	2
DRB1*0102	1	1.0	0	0.0	0	0	0	0.0	0	0
DRB1*1501	22	22.6	10	21.2	5	5	2	8.0	0	2
DRB1*1502	8	8.2	5	10.6	2	3	1	4.0	0	1
DRB1*1504	1	1.0	0	0.0	0	0	0	0.0	0	0
DRB1*1601	0	0.0	0	0.0	0	0	1	4.0	0	0
DRB1*1602	5	5.1	3	6.3	2	1	1	4.0	0	1
DRB1*0301	5	5.1	4	8.5	4	0	2	8.0	1	1
DRB1*0306	0	0.0	1	2.1	1	0	0	0.0	0	0
DRB1*0401	2	2.06	1	2.1	1	0	2	8.0	0	2
DRB1*0402	1	1.0	0	0.0	0	0	0	0.0	0	0
DRB1*0403	1	1.0	1	2.1	1	0	0	0.0	0	0
DRB1*0404	4	4.1	0	0.0	0	0	0	0.0	0	0
DRB1*0405	4	4.1	5	10.6	1	3[a]	5	20.0	2	3
DRB1*0406	2	2.06	1	2.1	1	0	4	16.0	1	3
DRB1*0410	1	1.0	0	0.0	0	0	1	4.0	0	0
DRB1*1101	10	10.3	1	2.1	0	1	4	16.0	2	2
DRB1*1104	6	6.1	0	0.0	0	0	0	0.0	0	0
DRB1*1201	5	5.1	4	8.5	2	2	1	4.0	0	1
DRB1*1202	13	13.4	7	14.8	3	2[a]	4	16.0	1	3
DRB1*1206	1	1.0	0	0.0	0	0	0	0.0	0	0
DRB1*1301	2	2.06	3	6.3	1	1[a]	0	0.0	0	0
DRB1*1302	1	1.0	2	4.2	1	0[a]	1	4.0	0	0
DRB1*1303	2	2.06	1	2.1	0	1	0	0.0	0	0
DRB1*1305	1	1.0	0	0.0	0	0	0	0.0	0	0
DRB1*1312	0	0.0	1	2.1	0	1	0	0.0	0	0
DRB1*1401	5	5.1	2	4.2	0	1[a]	1	4.0	0	1
DRB1*1403	2	2.06	2	4.2	1	0[a]	0	0.0	0	0
DRB1*1404	2	2.06	1	2.1	1	0	0	0.0	0	0
DRB1*1405	2	2.06	2	4.2	0	1[a]	1	4.0	0	1
DRB1*0701	32	32.9	10	21.2	6	4	3	12.0	0	2[a]
DRB1*0801	2	2.06	0	0.0	0	0	0	0.0	0	0
DRB1*0802	0	0.0	2	4.2	0	2	1	4.0	0	1
DRB1*0803	6	6.1	3	6.3	3	0	2	8.0	0	2
DRB1*0901	26	26.8	9	19.1	5	4	5	20.0	1	2[a]
DRB1*1001	3	3.09	1	2.1	1	0	1	4.0	0	1

[a] The number of individuals characterized for seroreactivity to *C. jejuni* may not necessarily add to the number of individuals with the particular allele due to the fact that not all HLA-typed cases were characterized for seroreactivity against *C. jejuni*.

Table II. *Distribution of HLA-DQB1 alleles among control, AMAN and AIDP cases, and* C. jejuni *seropositive and seronegative patients*

	Control		AMAN				AIDP			
Alleles	*n* (97)	%	*n* (46)	%	Sero +	Sero −	*n* (23)	%	Sero +	Sero −
DQB1*0201	6	6.3	6	13	5	1	2	8.6	1	1
DQB1*0202	24	25.2	8	17.3	4	3[a]	2	8.6	0	1[a]
DQB1*0301	41	43.1	16	34.7	8	7[a]	9	39.1	2	7
DQB1*0302	9	9.4	2	4.3	2	0	4	17.3	1	3
DQB1*0303	28	29.4	10	21.7	5	4[a]	7	30.4	1	3[a]
DQB1*0304	1	1	0	0.0	0	0	0	0.0	0	0
DQB1*0401	6	6.3	6	13	1	4[a]	5	21.7	2	3
DQB1*0402	2	2.1	2	4.3	0	2	2	8.6	0	1[a]
DQB1*0501	11	11.5	2	4.3	1	1	3	13	0	3
DQB1*0502	9	9.4	6	13	3	3	3	13	0	2[a]
DQB1*0503	7	7.3	4	8.6	1	1[a]	1	4.3	0	1
DQB1*0601	14	14.7	9	19.5	5	4	2	8.6	0	2
DQB1*0602	19	20	9	19.5	6	2[a]	1	4.3	0	1
DQB1*0603	2	2.1	2	4.3	1	1	0	0.0	0	0
DQB1*0604	1	1	1	2.1	1	0	0	0.0	0	0
DQB1*0605	0	0.0	1	2.1	0	0	0	0.0	0	0
DQB1*0609	0	0.0	1	2.1	0	0	0	0.0	0	0

[a] The number of individuals characterized for seroreactivity to *C. jejuni* may not necessarily add to the number of individuals with the particular allele due to the fact that not all HLA-typed cases were characterized for seroreactivity against *C. jejuni*.

Statistical analysis

Two-tailed Fisher's exact test (contingency table) was used to determine whether there was a statistical difference between the HLA frequencies of patients and controls using the Instat version 3.05 (GraphPad, San Diego, CA) statistical analysis program for the Windows 95. The *p* values were corrected, when appropriate, for the number of alleles involved. Additionally, differences in the distribution of HLA alleles between any of the groups compared (control, AMAN, and AIDP) was evaluated with a χ^2 method by *k* contingency tables, where *k* is the number of HLA alleles for a particular locus. This test was performed using the StatView software program for Windows version 4.5 (Abacus Concepts, Berkley, CA). This test provides an overall indication of whether there is a significant deviation in allele frequencies between any two groups compared and requires no correction for the number of alleles. The relative contribution of the DRβ/DQβ epitopes, which are in linkage disequilibrium, was evaluated by the method of Svjegaard and Ryder (36). Relative risk (rr) has been calculated as $rr = [Pd(1 - Pc)/(1 - Pd)Pc]$ where *Pd* and *Pc* are the frequencies of individuals positive for the allele or the epitope among patients and controls, respectively.

Serological studies for anti-campylobacter Abs

Abs to *Campylobacter jejuni* were measured using an isotype (IgG, IgA, IgM)-specific enzyme immunoassay as previously described (29). Patients were considered to have evidence of recent infection if the OD ratio was >2.0 in at least two Ig classes.

Table III. *Distribution of HLA-DPB1 alleles among control, AMAN and AIDP cases, and* C. jejuni *seropositive and seronegative patients*

	Control		AMAN				AIDP			
Alleles	*n* (90)	%	*n* (45)	%	Sero +	Sero −	*n* (22)	%	Sero +	Sero −
DPB1*0201	27	30	13	28.8	3	8[a]	8	36.3	5	0
DPB1*0202	6	6.6	6	13.3	4	2	2	9	1	1
DPB1*0301	6	6.6	6	13.3	3	2[a]	0	0.0	0	0
DPB1*0401	20	22.2	9	20	7	2	6	27.2	2	3[a]
DPB1*0402	9	10	8	17.7	6	2	1	4.5	0	1
DPB1*0501	52	57.7	28	62.2	13	10[a]	16	72.7	4	11[a]
DPB1*0901	3	3.3	1	2.2	1	0	0	0.0	0	0
DPB1*1001	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*1301	12	13.3	2	4.4	0	2	1	4.5	0	0[a]
DPB1*1401	6	6.6	1	2.2	0	1	1	4.5	1	0
DPB1*1601	1	1.1	1	2.2	1	0	0	0.0	0	0
DPB1*1701	11	12.2	5	11.1	3	1[a]	3	13.6	0	2[a]
DPB1*1901	0	0.0	1	2.2	0	1	0	0.0	0	0
DPB1*2201	0	0.0	1	2.2	0	1	0	0.0	0	0
DPB1*2202	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*2301	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*2401	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*3301	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*3601	2	2.2	0	0.0	0	0	0	0.0	0	0
DPB1*3801	3	3.3	0	0.0	0	0	0	0.0	0	0
DPB1*4101	1	1.1	0	0.0	0	0	1	4.5	0	0[a]
DPB1*4701	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*4801	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*5101	1	1.1	0	0.0	0	0	0	0.0	0	0
DPB1*5701	1	1.1	0	0.0	0	0	0	0.0	0	0

[a] The number of individuals characterized for seroreactivity to *C. jejuni* may not necessarily add to the number of individuals with the particular allele due to the fact that not all HLA-typed cases were characterized for seroreactivity against *C. jejuni*.

Table IV. *Differential distribution of the DQβ ED$^{70-71}$ and RPD$^{55-57}$ epitopes among control, AMAN patients, and AIDP patients*

	Control		AIDP		AMAN					
DQβ epitopes	*n* (97)	%	*n* (23)	%	*n* (46)	%	$p_{\text{control-AMAN}}$	$p_{\text{control-AIDP}}$	$\text{rr}_{\text{control-AIDP}}$	$p_{\text{AMAN-AIDP}}$
ED$^{70-71}$	8	8.4	7	30.4	8	17.5	0.153	0.009	3.7	0.231
RPD$^{55-57}$	40	42.1	4	17.3	20	43.4	0.856	0.05	0.4	0.036

Results

Control, AMAN, and AIDP cases were typed for identifying the DRB1*, DQB1*, and DPB1* alleles using DNA-based methodology. The total number of subjects typed and alleles identified are indicated under each category in Tables I–III. The frequencies of the different alleles of the three loci in the control population are in general agreement with previously published HLA class II typing in the same population of northern China (37).

Differential distribution of DQB1 amino acid epitopes among controls and the two forms of GBS

The different DQB1 alleles identified in the control, AMAN, and AIDP cases and their respective frequencies are shown in Table II. None of the alleles shows a statistically significant difference in the three comparisons (control/AMAN, control/AIDP, and AMAN/AIDP) using Fisher's exact test, particularly after the *p* values have been corrected for the number of alleles. Additionally, statistical analysis for detecting differences in allele frequency between any two of these groups has been performed with a χ^2 by *k* test, which provided the same results. However, in the control/AIDP comparison the DQB1* 0401 allele was significantly increased ($p = 0.03$) before the *p* value correction. This allele possesses a unique epitope (ED$^{70-71}$) that has been previously demonstrated on the DRβ molecule to have an important functional role in terms of both peptide binding and TCR recognition (38–40). Furthermore, positions DRβ^{70} and DRβ^{71} have been shown to be associated with tuberculoid leprosy (41) and rheumatoid arthritis (42–44), while position DPβ^{69} (equivalent to DRβ^{71}) has been demonstrated to be associated with beryllium hypersensitivity (BH) (45). The frequency of DQβ ED$^{70-71}$ was therefore evaluated in the control and AIDP populations. This analysis showed that DQβ ED$^{70-71}$ has a differential distribution in the two populations and is significantly increased among AIDP subjects ($p = 0.009$; rr = 3.7; Table IV).

The alleles DQB1*0503, DQB1*0601, DQB1*0602, and DQB1*0603 were decreased in AIDP cases compared with both controls and AMAN cases, although these differences were not statistically significant (Table II). These alleles are characterized by the unique epitope RPD$^{55-57}$ (Fig. 1). Since position 57 of the DQβ-chain has been implicated in disease susceptibility, for example, in insulin-dependent diabetes mellitus (46), it is possible that this DQβ position may be critical to other diseases as well. Analysis of the RPD$^{55-57}$ epitope was therefore performed and showed that this epitope was significantly reduced among AIDP cases compared with either control ($p = 0.05$) or AMAN ($p = 0.036$) cases (Table IV). When R^{55}, P^{56}, and D^{57} were analyzed as individual amino acids there were no significant differences among the populations studied.

The DQβ ED$^{70-71}$ epitope was positively associated with AIDP, whereas the RPD$^{55-57}$ epitope was negatively associated. The DQβ ED$^{70-71}$ is in linkage disequilibrium with another residue located at position 56 (L^{56}; Fig. 1) The DQB1 alleles in this population that are ED$^{70-71}$ are always L^{56}, and every L^{56}-positive DQB1 allele is also ED$^{70-71}$ positive (Fig. 1). It therefore appears that the DQβ L^{56}/ED$^{70-71}$ epitopes are positively associated with the disease, while the equivalent epitope RPD$^{55-57}$on other alleles is negatively associated with AIDP.

HLA-DRB1 and HLA-DPB1 allele distribution

DRB1 and DPB1 allele distribution was evaluated among the different groups of controls and patients (AMAN or AIDP; Tables I and III). None of the individual DRB1 alleles was associated with either AMAN or AIDP when evaluated by Fisher's exact test or the χ^2 by *k* tables. However, the DRB1* 04 alleles as an entity were increased among AIDP cases (corrected $p = 0.05$, correction includes 12 comparisons). The unique common epitope among the different DRB1*04 alleles that is different from the other DRB alleles is the sequence E^{9}V^{11}H^{13}. Upon re-evaluation of the distribution of this epitope among AIDP and control individuals, this epitope is significantly increased among AIDP patients (44% AIDP vs 15.4% controls; $p = 0.004$; rr = 2.85; Table V*A*). This epitope (E^{9}V^{11}H^{13}) was found to be in linkage disequilibrium with the DQβ ED$^{70-71}$ epitope in both the control (Table V*B*) and AIDP subjects (Table V*C*) studied. To determine whether either of the

```
                 1         10        20        30        40        50        60        70        80        90
                 |         |         |         |         |         |         |         |         |         |
DQB1*0501  RDSPEDFVYQ FKGLCYFTNG TERVRGVTRH IYNREEYVRF DSDVGVYRAV TPQGRPVAEY WNSQKEVLEG ARASVDRVCR HNYEVAYRGI
DQB1*0502  ---------- ---------- ---------- ---------- ---------- ------S--- ---------- ---------- ----------
DQB1*0503  ---------- ---------- ---------- ---------- ---------- ----RPD--- ---------- ---------- ----------
DQB1*0601  --P-----L- --AM------ -----Y---Y ------D--- ---------- ----RPD--- -----DI--R T--EL-T--- ------F---
DQB1*0602  --------F- ---M------ -----L---Y -------A-- ---------- ----RPD--- ---------- T--EL-T--- ------F---
DQB1*0603  ---------- ---M------ -----L---- -------A-- ---------- ----RPD--- ---------- T--EL-T--- ------F---
DQB1*0604  ---------- ---M------ -----L---- -------A-- ---------- ---------- ---------R T--EL-T--- -----G----
DQB1*0605  ---------- ---M------ -----L---Y -------A-- ---------- ---------- ---------R T--EL-T--- -----G----
DQB1*0609  ---------- ---M------ -----L---Y -------A-- ---------- ---------- ---------R T--EL-T--- -----G----
DQB1*0201  ---------- ---M------ -----L-S-S ------I--- -----EF--- -LL-L-A--- -----DI--R K--A------ ---QLEL-TT
DQB1*0202  ---------- ---M------ -----L-S-S ------I--- -----EF--- -LL-L-A--- -----DI--R K--A----- ----QLEL-TT
DQB1*0301  ---------- --AM------ -----Y---Y -------A-- ----E----- --L-P-D--- ---------R T--EL-T--- ---QLEL-TT
DQB1*0302  ---------- ---M------ -----L---Y -------A-- ---------- --L-P-A--- ---------R T--EL-T--- ---QLEL-TT
DQB1*0303  ---------- ---M------ -----L---Y -------A-- ---------- --L-P-D--- ---------R T--EL-T--- ---QLEL-TT
DQB1*0304  ---------- --AM------ -----Y---Y -------A-- ----E----- --L-P-A--- ---------R T--EL-T--- ---QLEL-TT
DQB1*0401  ---------F- ---M------ --L------Y -------A-- ---------- --L-RLD--- -----DI--E D-----T--- ---QLEL-TT
DQB1*0402  ---------F- ---M------ ---------Y -------A-- ---------- --L-RLD--- -----DI--E D-----T--- ---QLEL-TT
```

FIGURE 1. Amino acid sequences of DQB1 alleles identified in the population studied. Unique epitopes for the alleles of interest are shown as bold characters.

Table V. *Differential distribution of the DRβE^{9}V^{11}H^{13} epitope among control subjects and AIDP patients (A), and association between the DQβED$^{70-71}$ epitope and the DRβE^{9}V^{11}H^{13} epitope in the control population (B) and AIDP patients (C)*

A.	Epitope	Control *n* (97)	Control %	AIDP *n* (25)	AIDP %	*p*	rr
	DRβE^{9}V^{11}H^{13}	15	15.4	11	44	0.004	2.85

B.		DQβED$^{70-71}$ +	DQβED$^{70-71}$ −
	DRβE^{9}V^{11}H^{13} +	5	10
	DRβE^{9}V^{11}H^{13} −	3	79
		$p = 0.002$	

C.		DQβED$^{70-71}$ +	DQβED$^{70-71}$ −
	DRβE^{9}V^{11}H^{13} +	7	4
	DRβE^{9}V^{11}H^{13} −	0	14
		$p = 0.0007$	

two epitopes, DRβ E^{9}V^{11}H^{13} or DQβ ED$^{70-71}$, had a stronger association with AIDP, the DRβ E^{9}V^{11}H^{13}-positive/DQβ1 ED$^{70-71}$-negative individuals were compared with DRβ E^{9}V^{11}H^{13}-negative/DQβ ED$^{70-71}$-positive individuals in the AIDP and control subjects, according to Svejgaard and Ryder (36). It was determined that neither of the two epitopes had a stronger association ($p = 0.5$) over the other. No significant associations were identified with any DPB1 allele or epitope.

Seroreactivity to C. jejuni *and association with HLA class II alleles/epitopes*

Tables I, II, and III show the number of individuals characterized for their seroreactivity to *C. jejuni* who are positive with particular DRB1, DQB1, and DPB1 alleles. When the seroreactivity status was evaluated in relationship to the HLA DRB1, DQB1, or DPB1 alleles or the RLD$^{55-57}$/ED$^{70-71}$ and RPD$^{55-57}$ DQβ epitopes among AIDP or AMAN patients, no significant correlation was established. No association was identified between seroreactivity status and DQ3 specificity in this Chinese population, as was suggested in studies on *C. jejuni*-associated GBS in European patients with AIDP (28). The lack of association in our population must be considered in light of the small population sampled in our study.

Discussion

Elucidation of the pathogenesis of GBS has been hampered by the multifactorial nature of the illness. The discovery that GBS is not a single entity, but a syndrome that can be classified into types based on different pathologic characteristics suggested that different mechanisms of disease might be operative. Careful selection and characterization of patients by subtype of GBS, AIDP and AMAN, provided the base for an independent evaluation of the host-related factors that may be responsible for disease susceptibility and development. The DNA-based typing of the HLA class II alleles in control, AMAN, and AIDP individuals reveal that HLA class II epitopes are not distributed equally in the three groups, and certain DQβ/DRβ epitopes were associated with AIDP. The lack of any associations between controls and the AMAN group strongly suggests that the mechanism of disease susceptibility is different between AMAN and AIDP. This is in accord with previous histological and immunological studies that demonstrate different histologic appearances of the two types of GBS (14, 15), different targets of complement-mediated Ab attack on the nerve in AIDP and AMAN (6), and different Ab responses in AIDP and AMAN (9).

The specific epitopes identified as disease protection is the DQβ RPD$^{55-57}$ and susceptibility are the DQβ L^{56}/ED$^{70-71}$/DRβE^{9}V^{11}H^{13} epitopes, respectively. There are a number of interesting points with respect to these epitopes and their role in AIDP.

The RPD$^{55-57}$ epitope is associated with protection; however, the individual residues R, P, and D, when evaluated independently, are not. This is reminiscent of the association of rheumatoid arthritis with the residues DRβ^{67}, DRβ^{70}, DRβ^{71}, and DRβ^{74} together, but not any of these residues independently (42). Crystallography of DR molecule revealed that the side chains of DRβ residues 70, 71, and 74 participate in the formation of pocket 4, and as such influence the kind of peptides that can be accommodated in the binding grove of DR molecules. More recently, it has been proposed that this DRβ$^{70-74}$ peptide is an appropriate peptide for binding to DQ molecules that influence T cell selection and as such determine autoreactivity (47). Regardless of the role of this DR epitope, whether it is part of a binding pocket of an HLA molecule or part of a peptide bound to an HLA molecule, this epitope operates as a defined sequence and not as individual residues. The RPD$^{55-57}$ epitope of this study is also located in a position that is known to influence peptide binding and T cell recognition. Residue 57 participates in the formation of a pocket 9 of DQ molecules and is associated with susceptibility to insulin-dependent diabetes mellitus. Furthermore, studies involving transgene NOD mice with I-A molecules modified at position 56 has been demonstrated to influence insulitis (48–50). It is therefore likely that this epitope (RPD$^{55-57}$) has an important functional role as a cassette and, as such, determines and influences binding of peptides in the DQ molecules and influences processes that result in protection from AIDP.

The L^{56}/ED$^{70-71}$ epitope of susceptibility is unclear as to whether it includes two independent elements of susceptibility the L^{56} and the ED$^{70-71}$ or one that includes both. Based on the observation that the RPD$^{55-57}$ peptide has a protective effect, it is not unreasonable to hypothesize that the L^{56} or RLD$^{55-57}$ peptide located in the equivalent position may have a susceptibility role. The fact that L^{56} and ED$^{70-71}$ are tightly linked suggest that these two epitopes on the DQB1*04 alleles have a functional role, and it is therefore likely that the susceptibility effect is caused by a coordinated function of both. In a recent study (45) it was observed that the DPβ D^{55} position was associated with BH. Additionally the DPB1 alleles that were D^{55} positive and associated with BH were also D^{69} positive, which is another residue strongly associated with BH. These two positions are not in linkage disequilibrium, and the implication is that D^{69} is the primary element of susceptibility to BH, while D^{55} is an independent and additional element of susceptibility to BH. This may suggest a coordination between these two positions that predisposes to BH. Considering that DPβ^{55} and DPβ^{69} are the equivalent positions of DQβ^{57} and DQβ^{71}, the L^{56} or RLD$^{55-57}$/ED$^{70-71}$ epitopes on DQβ molecules may work together to produce a cumulative new effect.

The linkage disequilibrium between the DRβ $E^9V^{11}H^{13}$ and DQβ ED^{70-71} epitopes and the small sample size of AIDP patients makes difficult to distinguish their possible contribution to AIDP. It should be noted that the linkage disequilibrium between the DR and DQ epitopes observed is partially due to the linkage disequilibrium of the DRB1*0405 with DQB1*0401 alleles ($p = 0.0001$; odds ratio, 333), both of which include the DRβ $E^9V^{11}H^{13}$ and DQβ ED^{70-71} epitopes, respectively. Linkage disequilibrium involving the same alleles has also been reported in the same population of northern Chinese by Gao et al. (37).

The relative significance, therefore, of these two epitopes for disease susceptibility is not known. However, DRβ $E^9V^{11}H^{13}$ potentially can play a significant role because residues 9, 11, and 13 participate in the formation of pockets 9, 6, and 4, respectively. This epitope can be important either as an epitope that influences peptide binding to DR4 alleles and therefore TCR interactions or as an epitope that may be presented by other class II molecules to influence T cell thymic selection. This second alternative has been proposed for the association of DRB1 and DQB1 alleles with rheumatoid arthritis, in which certain HLA-DRβ epitopes, when present in the context of certain HLA-DQB1 alleles, appear to influence disease progression (47, 51).

The possibility always remains that the DRβ-DQβ haplotype that includes these epitopes is in linkage disequilibrium with another gene(s) within the MHC that modulates the immune response and influences disease development. It is interesting that the DRB1*0405-DQB1*0401 haplotype has been reported to be present in the northern Chinese population and not in other ethic groups (52). The significance of another MHC gene(s) for the disease can be evaluated because the complete sequence and gene map of the human MHC has been published (53). A number of studies recently concentrates on evaluating the frequencies of microsatellite markers within the MHC to determine the involvement of other genes, besides the HLA molecules, in disease associations (54, 55).

Antecedent infections are common triggering events in the development of GBS, and gastroenteritis with *C. jejuni* is the most identifiable infectious disease associated with GBS (18). Rees et al. (28) identified DQB1*03 as being associated with *Campylobacter*-positive GBS patients compared with seronegative patients; however, Yuki et al. (56) did not confirm this in a different population. Our studies also did not find an allele association in either AIDP or AMAN with evidence of prior *Campylobacter* infection. The power of analysis is insufficient to make a definite statement, however, due to the fact that we had a small number of *Campylobacter*-seropositive cases in the AIDP group. Furthermore, alternative mechanisms may be operative in the AMAN form of GBS. AMAN is associated with *Campylobacter* infection (18), and patients with AMAN develop anti-ganglioside Abs, such as anti-GD1a, putatively directed against ganglioside-like structures in the *Campylobacter* lipo-oligosaccharide (29). Anti-ganglioside Abs produced in response to *Campylobacter* have been shown to react with neuronal gangliosides (57). AMAN may be elicited through a different mechanism, and, therefore, an HLA class II association may be absent as defined in our study.

The association of the epitopes that are positively and negatively associated with AIDP is further supported by studies showing that the same epitopes are instrumental and critical for peptide binding and T cell recognition (38–40). These results further underline the significance of these epitopes as being relevant and important to the functioning of HLA molecules and form the basis for our understanding of the pathophysiology of certain diseases. Identification of the other participating factors involved, such as Ags and T cell responses, will contribute significantly to our understanding of these processes and form the basis for intervention therapies.

References

1. Ho, T. W., G. M. McKhann, and J. M. Griffin. 1998. Human autoimmune neuropathies. *Annu. Rev. Neurosci. 21:187.*
2. Alter, M. 1990. The epidemiology of Guillain-Barré syndrome. *Ann. Neurol. 27(Suppl.):S7.*
3. Peterman, A. F., D. D. Daly, F. R Dion, and H. M Keith. 1959. Infectious neuronitis (Guillain-Barré syndrome) in children. *Neurology 9:533.*
4. Asbury, A. K., B. G. Arnason, and R. D. Adams. 1969. The inflammatory lesion in idiopathic polyneuritis. *Medicine 48:173.*
5. Prineas, J. W. 1972. Acute idiopathic polyneuritis: an electron microscope study. *Lab. Invest. 26:133.*
6. McKhann, G. M., D. R. Cornblath, J. W. Griffin, T. W. Ho, C. Y. Li, Z. Jiang, H. S. Wu, G. Zhaori, Y. Liu, L. P. Jou, et al. 1993. Acute motor axonal neuropathy: a frequent cause of acute flacid paralysis in China. *Ann. Neurol. 33:333.*
7. Griffin, J. W., C. Y. Li, T. W. Ho, M. Tian, C. Y. Gao, P. Xue, B. Mishu, D. R. Cornblath, C. Macko, G. M. McKhann, et al. 1996. Pathology of the motor-sensory axonal Guillain-Barré syndrome. *Ann. Neurol. 39:17.*
8. Fisher, M. 1956. An unusual variant of acute idiopathic polioneuritis (syndrome of ophthalmoplegia ataxia and areflexia). *N. Engl. J. Med. 255:57.*
9. Griffin, J. W., C. Y. Li, T. W. Ho, P. Xue, C. Macko, C. Y. Gao, C. Yang, M. Tian, B. Mishu, and D. R. Cornblath. 1995. Guillain-Barré syndrome in northern China: the spectrum of neuropathologic changes in clinically defined cases. *Brain 118:577.*
10. Arnason, B. G. W., and B. Soliven. 1993. Acute inflammatory demyelinating polyradiculopathy. In: *Peripheral Neuropathy*. P. J. Dyck, P. K. Thomas, J. W. Griffin, P. A. Low, and J. F. Poduslo, eds. Saunders, Philadelphia, p. 1437.
11. Hartung, H. P., G. Stoll, and K. V. Toyka. 1993. Immune reactions in the peripheral nervous system. In: *Peripheral Neuropathy*. P. J. Dyck, P. K. Thomas, J. W. Griffin, P. A. Low, and J. F. Poduslo, eds. Saunders, Philadelphia, p. 418.
12. Hartung, H. P., J. D. Pollard, G. K. Harvey, and K. V. Toyka. 1995. Immunopathogenesis and treatment of the Guillain-Barré syndrome. Part I. *Muscle Nerve 18:137.*
13. Hafer-Macko, C., K. A. Sheikh, C. Y. Li, T. W. Ho, D. R. Cornblath, G. M. McKhann, A. K. Asbury, and J. W. Griffin. 1996. Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann. Neurol. 39:625.*
14. Feasby, T. E., J. J. Gilbert, W. F. Brown, C. F. Bolton, A. F. Hahn, W. F. Koopman, and D. W. Zochodne. 1986. An acute axonal form of Guillain-Barré polyneuropathy. *Brain 109:1115.*
15. Feasby, T. E., A. F. Hahn, W. F. Brown, C. F. Bolton, J. J. Gilbert, and W. J. Koopman. 1993. Severe axonal degeneration in acute Guillain-Barré syndrome: evidence of two different mechanisms? *J. Neurol. Sci. 116:85.*
16. Hafer-Macko, C., S. T. Hsieh, C. Y. Li, T. W. Ho, K. A. Sheikh, D. R. Cornblath, G. M. McKhann, A. K. Asbury, and J. W. Griffin. 1996. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann. Neurol. 40:635.*
17. Scully, R., E. J. Mark, W. F. McNeely, S. H. Ebeling, L. D. Phillips, and S. M. Ellender. 1999. Case 39-1999. *N. Engl. J. Med. 26:1996.*
18. Nachamkin, I., B. M. Allos, and T. W. Ho. 2000. *Campylobacter jejuni* infection and the association with Guillain-Barré syndrome. In: *Campylobacter*, 2nd Ed. I. Nachamkin and M. J. Blaser, eds. ASM Press, Washington, D.C., p. 155.
19. Yuki, N., S. Handa, T. Taki, T. Kasama, M. Takahashi, and K. Saito. 1992. Cross-reactive antigen between nervous tissue and a bacterium elicits Guillain-Barré syndrome: molecular mimicry between ganglioside GM1 and lipopolysaccharide from Penner's serotype 19 *Campylobacter jejuni*. *Biomed. Res. 13:451.*
20. Yuki, N., T. Taki, F. Inagaki, T. Kasama, M. Takahashi, K. Saito, S. Handa, and T. Miyatake. 1993. A bacterium lipopolysaccharide that elicits Guillain-Barré syndrome has a GM1 ganglioside-like structure. *J. Exp. Med. 178:1771.*
21. Tauxe, R. V. 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In *Campylobacter jejuni: Current Status and Future Trends*. I. Nachamkin, M. J. Blaser, and L. S. Tompkins, eds. American Society for Microbiology, Washington, D.C., pp. 9–19.
22. Sheikh, K. A., I. Nachamkin, T. W. Ho, H. J. Willison, J. Veitch, B. S. Ung, C. Y. Li, B. G. Shen, D. R. Cornblath, A. K. Asbury, et al. 1998. *Campylobacter jejuni* lipopolysaccharides in Guillain-Barré syndrome: molecular mimicry and host susceptibility. *Neurology 51:2.:371* .
23. Stewart, G. J., J. D. Pollard, J. G. McLeod, and C. M. Wolnizer. 1978. HLA antigens in the Landry-Guillain-Barré syndrome and chronic relapsing polyneuritis. *Ann. Neurol .4:285.*
24. Latovitzki, N., N. Suciu Foca, A. S. Penn, M. R. Olarte, and A. M. Chutorian. 1979. HLA typing and Guillain-Barré syndrome. *Neurology 29:743.*
25. Kaslow, R. A., J. Z. Sullivan-Bolyai, B. Hafkin, L. B. Schonberger, L. Kraus, M. J. Moore, E. Yunis, and R. M. Williams. 1984. HLA antigens in Guillain-Barré syndrome. *Neurology 34:240.*
26. Winer, J. B., D. Briggs, K. Welsh, and R. A. C. Hughes. 1988. HLA antigens in the Guillain Barré syndrome. *J. Neuroimmunol. 18:1.:13.*
27. Gorodezky, C., B. Varela, L. E. Castro-Escobar, Chavez-Negrete, A. Escobar-Gutierrez, and Martinez-Mata, J. 1983. HLA-DR antigens in Mexican patients with Guillain-Barré syndrome. *J. Neuroimmunol. 4:1.*
28. Rees, J. H., R. W. Vaughan, E. Kondeatis, and R. A. C. Hughes. 1995. HLA-class II in Guillain-Barré syndrome and Miller Fisher syndrome and their association with preceding *Campylobacter jejuni* infection. *J. Neuroimmunol. 62:53.*
29. Ho, T. W., H. Willison, I. Nachamkin, C. Y. Li, J. Veitch, H. Ung, G. R. Wang, R. C. Liu, D. R. Cornblath, A. K. Asbury, et al. 1999. Anti-GD1a antibody

distinguishes axonal from demyelinating forms of Guillain-Barré syndrome. *Ann. Neurol. 45:168.*
30. Asbury, A. K., and D. R. Cornblath. 1990. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann. Neurol. 27(Suppl.):S21.*
31. Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques 10:506.*
32. Vaughan, R. W., J. S. Lanchbury, S.G. Marsh, M. A. Hall, J. G. Bodmer, and K. I. Welsh. 1990. The application of oligonucleotide probes to HLA class II typing of the DRB sub-region. *Tissue Antigens 36:149.*
33. Obata, F., K. Ito, T. Kaneko, Y. G. Yang, K. Onda, I. Ito, N. Yabe, K. Watanabe, and N. Kashiwagi. 1991. HLA-DR gene frequencies in the Japanese population obtained by oligonucleotide genotyping. *Tissue Antigens 38:124.*
34. Bugawan, T. L., A. B. Begovich, and H. A, Erlich. 1990. Rapid HLA-DPB typing using enzymatically amplified DNA and nonradioactive sequence-specific oligonucleotide probes. *Immunogenetics 32:231.*
35. Ballas, M., G. Oechsle, T. H. Eiermann, C. Muller, A. Wopl, and S. F. Goldmann. 1992. A DRB oligonucleotide typing system defining 50 of 56 DRB alleles. In: *Proceedings of the Eleventh International Histocompatibility Workshop and Conference*. K. Tsuji, M. Aizawa, and T. Sasazuki, eds. Oxford University Press, New York, p. 311.
36. Svejgaard, A., and L. P. Ryder. 1994. HLA and disease associations: detecting the strongest association. *Tissue Antigens 43:18.*
37. Gao, X., Y. Sun, J. An, M. Fernandez-Vina, J. Qou, L. Lin, and P. Stastny. 1991. DNA typing for HLA-DR, -DQ, and -DP alleles in a Chinese population using the polymerase chain reaction (PCR) and oligonucleotide probes. *Tissue Antigens 38:24.*
38. Hammer, J., F. Gallazzi, E. Bono, R. W. Karr, J. Guenot, P. Valsasnini, Z. A. Nagy, and R. Sinigaglia. 1995. Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. *J. Exp. Med. 181:1847.*
39. Fu, X. T., C P. Bono, S. L. Woulfe,. C. Swearingen, N. L. Summers, R. Sinigagalia, A. Sette, B. D. Schwartz, and R. W. Karr. 1995. Pocket 4 of the HLA-DR (α,β1*0401) molecule is a major determinant of T cells recognition of peptide. *J. Exp. Med. 181:915.*
40. McNicholl, J. M., W. C. Whiteworth, F. Oftung X. Fu, T. Shinnick, P. E. Jensen, M. Simon, R. M. Wohlhueter, and R. W. Karr. 1995. Structural requirements of peptide and MHC for DRα,β 1*0401-restricted T cell antigen recognition. *J. Immunol. 15:1951.*
41. Zerva, L., B. Cizman, N. K. Mehra, S. K. Alahri, R. Murali, C. M. Zmijewski, M., Kamoun, and D. S. Monos. 1996. Arginine at positions 13 or 70–71 in pocket 4 of HLA-DRB1 alleles is associated with susceptibility to tuberculoid leprosy. *J. Exp. Med. 183:829.*
42. Gregersen, P. K., J. Silver, and R. J. Winchester. 1987. The Shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum. 30:1205.*
43. Nepom, G. T. 1995. Class II antigens and disease susceptibility. *Annu. Rev. Med. 46:17.*
44. Zanelli, E., F. C. Breedveld, R. R. P. de Vries. 2000. HLA class II association with rheumatoid arthritis: facts and interpretations. *Hum. Immuol. 61:1254.*
45. Rossman, M. D., J. Stubbs, W. W. Lee, E. Argyris, E. Magira, and D. Monos. 2002. HLA Amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *Am. J. Respir. Crit. Care Med. 165:788.*
46. Todd, J., J. I. Bell, and H. O McDevitt. 1988. HLA antigens and insulin-dependent diabetes. *Nature 333:710.*
47. Zanelli, E., C. J. Krco, J. M Baisch, S. Cheng, and C. S. David. 1996. Immune response of HLA-DQ8 transgenic mice to peptides from the third hypervariable region of HLA-DRB1 correlates with predisposition to rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA 93:1814.*
48. Miyazaki, T., M. Uno, M. Uehira, H. Kikutani, T. Kishimoto, M. Kimoto, H. Nishimoto, J. Miyazaki, and K. Yamamura. 1990. Direct evidence for the contribution of the unique I-ANOD to the development of insulitis in non-obese diabetic mice. *Nature 345:722.*
49. Slattery, R. M., L. Kjer-Nielsen, J. Allison, B. Charlton, T. E. Mandel, J. F. A. P. Miller. 1990. Prevention of diabetes in non-obese diabetic I-A^k transgenic mice. *Nature 345:724.*
50. Lund, T., L. O'Reilly, P. Hutchings, O. Kanagauva, E. Simpson, R. Gravely, P. Chandler, J. Dyson, J. K. Picard, A. Edwards, et al. Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A β-chain or normal I-E α-chain. *Nature 345:727.*
51. Das, P., D. S., D. S. Bradlley, A. Geluk, M. M. Griffiths, H. S. Luthra, C. S. David. 1999. HLA-DRB1*0402 derived peptide (HV3 65–79) prevents collagen-induced arthritis in HLA-DQ8 transgenic mice. *Hum. Immunol. 60:575.*
52. Fernandez-Vina, M. A., X. Gao, M. E. Moraes, J. R. Moraes, I. Salatiel, S. Miller, J. Tsai, Y. Sun, J. An, Z. Layrisse, et al. 1991. Alleles at four HLA class II loci determined by oligonucleotide hybridization and their associations in five ethic groups. *Immunogenetics 34:299.*
53. MHC Sequencing Consortium. 1999. Complete sequence and gene map of a human major histocompatibility complex. *Nature 401:829.*
54. Monos, D. S., M. Kamoun, I. A. Udalova, E. Csanky, B. Cizman, R. L. Tutetskaya, J. B. Smirnova, V. G. Zharkov, D. Gasser, C. M. Zmijewski, et al. 1995. Genetic polymorphism of the human tumor necrosis factor region in insulin-dependent diabetes mellitus: linkage disequilibrium of TNFab: microsatellite alleles with HLA haplotypes. *Hum. Immunol. 44:70.*
55. Matsuzaka Y., S. Makino, K. Nakajima, M. Tomizawa, A. Oka, M. Kimura, S. Bahram, G. Tamiya, and H. Inoko. 2000. New polymorphic microsatellite markers in the human MHC class II region. *Tissue Antigens 56:492.*
56. Yuki, N., M. Takahashi, Y. Tagawa, K. Kashiwasw, K. Tadokoro, and K. Saito. 1997. Association of *Campylobacter jejuni* serotype and antiganglioside antibody in Guillen-Barré syndrome and Fisher's syndrome. *Ann. Neurol. 42:28.*
57. Goodyear, C. S. O'Hanlon, G. M. Plomp, J. J. Wagner, E. R. Morrison, I. Veitch, J. Cochrane, L. Bullens, R. W. M. Molenaar, P. C. Conner, et al. 1999. Monoclonal antibodies raised against Guillain-Barré syndrome-associated *Campylobacter jejuni* lipopolysaccharides react with neuronal gangliosides and paralyze muscle-nerve preparations. *J. Clin. Invest. 104:697.*