Immuno - Rheum

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GENETICS AND AUTOIMMUNITY

by

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These notes were prepared to accompany a lecture given by the author on May 20, 1982. They are largely based on two manuscripts which are to be published elsewhere. The first is entitled "HLA-D, HLA-DR and Other Ia-like Antigens in Man", by Peter Stastny, Edward J. Ball, Gabriel Nunez and Jun Okada, and is currently in press. The second is entitled "Rheumatoid Arthritis: relationship with HLA-D" and is being submitted for publication. To complement this material, a table summarizing most of the important associations of diseases with HLA/DR has also been included. As is customary the copyrights have or will be assigned to the publishers and reproduction of this material, without permission, is therefore prohibited.

HLA-D, HLA-DR AND OTHER IA-LIKE ANTIGENS IN MAN.

INTRODUCTION

The region of the main histocompatibility complex (MHC) that codes for the human Ia-like antigens is called HLA-D. The Ia antigens have been difficult because of their limited expression in only certain kinds of cells, and their preponderant manifestation in functional and cellular assays, rather than being ubiquituous targets like the other common transplantation antigens.

The importance of the Ia-like antigens stems from their role in the genetic control of the immune response, their associations with a variety of diseases and their probable role in organ transplantation. The study of the human Ia-like antigens has rapidly become one of the most active areas of contemporary biology.

However, the state of our understanding of the human Ia-like antigens can be appropriately described as primitive. Serologic methods allow the detection of one series of alloantigens, the HLA-DR antigens. In addition there are some broad "supertypic" antibodies and some antibodies that react with probable Ia-like antigens in monocytes and subsets of T lymphocytes. Everything else is based on cellular methods, especially primary and secondary mixed lymphocyte culture (MLC).

The cellular methods for typing for HLA-D were developed in 1973, and the efforts of the 1975 International Histocompatibility Workshop culminated with the recognition of the specificities Dw1 through Dw8. Although the specificities were upheld two years later with the discovery of the D-related (DR) antigens in close correspondence, many investigators have expressed the view that the mixed lymphocyte culture (MLC) determinants, detectable in primary MLC, appear to show greater complexity than can be adequately resolved with presently available methods. The analysis of secondary MLC using primed lymphocytes, has been even more complicated, suggesting the existence of stimulating determinants coded by other HLA loci and possibly also by loci which are not associated with the HLA chromosomal region.

Since the HLA-DR specificities were defined, it was observed that they were closely associated with HLA-D. However, the association was never complete. This suggested to some investigators that D and DR were separate entities, probably coded by separate loci. Other investigators, however, were unwilling to make conclusions about the genetics of HLA-D and DR, in view of obvious difficulties in the interpretation of the data, and because little true genetic information was available.

Family studies showing recombination and indicating that the D and DR determinants segregate independently, would be needed to demonstrate that separate loci are involved. Such recombinations might be expected to be quite rare if the presumed loci are separated by a very short distance.

A further difficulty stems from lymphocyte stimulation due to other ares of the HLA chromosomal region. Such stimulation may give rise to "false negative" HLA-D assignments. Also, cells from some individuals may fail to respond in MLC, although they do not have the corresponding antigen, giving rise to "false positive" results. One must remember that the positive assignment is based on a negative response.

Moreover, the serologic data presents complexities that need to be fully resolved. The specificity DRw6 is complicated and not fully understood. The supertypic specificities MT1, MT2, MT3 and MB1, MB2, MB3 can be interpreted in different ways and have cause serologic confusion due to the presence of multiple antibodies in the same serum. Additional complexities come from recently developed monocolonal antibodies against HLA-DR antigens. They suggest further heterogeneity, the possible existence of isotypes of DR antigens, and complications resulting from random association of polypeptide chains, giving rise to hybrid molecules.

TYPING FOR HLA-D BY MIXED LYMPHOCYTE CULTURE (MLC) WITH HOMOZYGOUS TYPING CELLS (HTC).

The fact that lymphocytes from HLA identical siblings do not stimulate each other, and usually give results that are similar to the values obtained in autologous controls, indicated that most of the lymphocyte stimulating activity, which is detectable in primary MLC is coded by genes of the HLA chromosome region (1). Exceptions to this rule were explainable as being the result of chromosomal recombination and led to the mapping of the main lymphocyte stimulating genes in a region centromeric to HLA-B (2). The low MLR response as an indication of identity at the main lymphocyte activating locus was found, subsequently, to work also when the donor of the stimulating cells had inherited identical lymphocyte activating determinants from both parents (3). Such homozygotes were found not to stimulate any of the family members who had inherited the same chromosomes and also some unrelated individuals who apparently possessed similar lymphocyte activating determinants (4). These observations served as the basis for the use of homozygous typing cells (HTC) as reagents for typing for the lymphocyte activating determinants. Technology was developed for freezing the HTCs for repeated use in typing experiments locally and also for distribution to other investigators.

The first extensive international exchange of HTCs was carried out under the auspices of the Sixth International Histocompatibility Workshop, in 1975 (5). In this workshop the reactions of typing cells against the panel were analyzed using two by two tables, and clustering of correlation coefficients, much the same way as HLA serum reactions are usually analyzed. As a result of this analysis, eight determinants were identified and given numerical designations. The locus coding for these antigens was given the name HLA-D.

In the performance of the actual MLC typing experiments, results are usually expressed as relative responses (4) by the method of the 75th percentile (6). It is customary, in the normalization of the data, to take into account both the ability of different responders to react and the variation in the ability of different stimulators to stimulate. The values obtained in this type of computation have sometimes been called "double normalized values" (7).

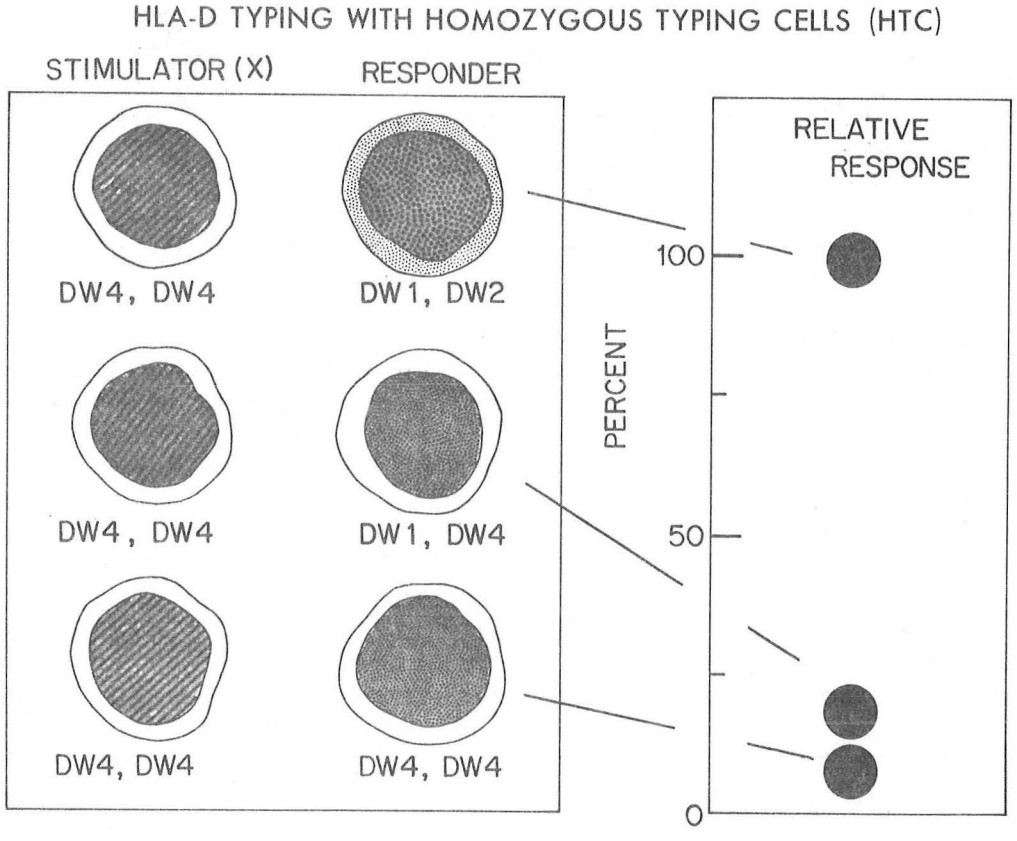

Figure 1. HLA-D typing with homozygous typing cells (HTC).

The basis of HTC typing is schematically illustrated in Figure 1. The mixed lymphocyte culture is made undirectional by irradiation of the stimulating cells, which in the case of the example are homozygous Dw4. The first responder, which lacks this antigen, gives a relative response of around 100%. The other two responders shown, one heterozygous and the other homozygous Dw4, recognize the Dw4 determinant as very similar to self, and give low reactions with relative responses of about 20%.

These "typing reactions" are usually not entirely negative and their upper limits may vary somewhat in different experiments and with different HTCs. It is difficult, therefore, to establish definite cut-off levels and a variety of methods have been used to attempt to utilize objective criteria for deciding whether a given cell donor, producing reactions which are not clearly negative or positive, should be considered to have the antigen in question. None of these methods appear to be entirely satisfactory and it seems best, in our experience, to consider some individuals to be untypable for technical reasons, rather than to make an arbitrary assignment.

The cause of these intermediate reactions is not entirely clear. There are probably more than one. It is well known that X-irradiation, at the levels commonly used in these experiments, although adequate for suppressing DNA synthesis, may not stop other events associated with antigen recognition by the lymphocyte. Such cells have been found to be able to secrete lymphocyte activating factors, which increase the level of DNA synthesis in the responding cells. This phenomenon has been called "back stimulation" (8) and is observed when the responder is heterozygous and not when both cells are homozygous for the same determinant.

In addition, it is now generally recognized that the products of HLA-D are not the only lymphocyte stimulating determinants and therefore, it appears likely that intermediate reactions may result from the effect of stimulation by products of these other loci. For instance, it has been repeatedly observed that cells from sibs who have a recombination between HLA-A and B, and who are mismatched only in the HLA-A region, give rise to some lymphocyte stimulation to a level of approximately 25% (9). It is not known whether the activation is due to HLA-A locus products themselves, or to products of an as yet not well defined second mixed lymphocyte culture stimulating locus, situated in the proximity of HLA-A.

Recently, several groups of investigators have described HLA linked lymphocyte activating determinants which are detectable most clearly when the responding lymphocytes are first primed (10,12). However, at least some of these lymphocyte activating

determinants, which are observed in secondary reactions with primed cells, also have recently been observed to have an influence in primary mixed lymphocyte cultures (13).

TYPING FOR HLA-D BY SECONDARY MIXED LYMPHOCYTE CULTURE USING PRIMED LYMPHOCYTES.

The secondary-type response to allogeneic antigens was studied by Andersen and Hayry (14) and subsequently by other investigators (15). While the primary mixed lymphocyte culture proliferative response peaks at 6 or 7 days after initiation of culture, secondary cultures peak after only 3 or 4 days. This early response could be demonstrated by measuring the DNA synthesis in proliferating lymphocytes and also by cell mediated cytotoxicity against donor type target cells. The possibility of utilizing the secondary reaction as a method for typing for the allogeneic stimulating determinants was proposed by Sheehy and coworkers (16). The method appeared attractive because it consisted of a positive assay to detect the presence of the antigens, and because the period of culture could be shortened to only 2 or 3 days. The idea was that primed lymphocytes would be frozen and utilized as reagents. Unfortunately, primed lymphocyte typing was found to be much more complicated than initially expected. At this writing, relatively few laboratories have succeeded to make this procedure a useful typing method.

There is little doubt that with certain selected primed cells, it is possible to obtain reactions which coincide quite closely with the definition of the HLA-D determinants. More frequently, however, the secondary response appears to include reactions to HLA-DR (16) and to other antigens, revealing a complexity of responses which has been quite resistant to analysis.

One interpretation of the puzzling array of reactions obtained with primed lymphocytes is that the primary MLC is relatively simple because only the strongest antigens give rise to an important response. During priming, on the other hand, clones of lymphocytes capable of recognizing weaker activating determinants are expanded, and so, the primed lymphocyte method is capable of detecting a variety of weaker antigens. In a subsequent section we will discuss a series of antigens which are HLA linked, but distinct from HLA-D, and are detectable with primed lymphocytes.

TYPING FOR THE HLA-D RELATED (DR) ANTIGENS

Many different investigators made preliminary observations which revealed the existence of a separate set of alloantibodies which reacted preferentially with B lymphocytes (18-20). Such antibodies reacted with the Daudi cell line, known not to

display any HLA-A, B or C determinants (18). They reacted with cells pretreated with turkey anti-human Beta 2 microglobulin (21), and they were detectable in sera which had been subjected to exhaustive platelet absorption (22). Preliminary work had suggested that such antibodies might have a relationship with HLA-D, because they were capable of inhibiting stimulation in mixed lymphocyte cultures (23).

These efforts culminated in the Seventh International Histocompatibility Workshop (24), in 1977, with the definition of eight specificities which appear to be closely correlated with the first eight HLA-D specificities. The antigens were defined by classical serologic methods of analysis and their relationship with the HLA-D antigens was so striking that it was relatively easy to assign numbers to them that would correspond to those of the HLA-D alleles.

Standard methods for typing for these antigens were developed which consisted of isolating the B lymphocytes either physically, by rosetting of T cells or by nylon wool filtration, or morphologically, by tagging them with an immuno-fluorescent marker as in the two-color immuno-fluorescence technique (25). The typing reagents are allo-antisera, which are usually platelet absorbed to remove antibodies against the HLA-A,B,C determinants. The method of detection most commonly used is a microcytotoxicity technique.

HLA-D/DR ANTIGENS IN DIFFERENT ETHNIC GROUPS

It is well known that HLA gene frequencies and relationships vary in different ethnic groups (26). The major northern European haplotypes A1, B8, DR3 and A3, B7, Dw2, DR2 decrease in frequency as one moves from north to south and they do not exist at all in Negro or in Mongoloid populations. It was not too surprising therefore to find that the very clear relationship between D and DR, in northern European populations, did not hold for some of the other ethnic groups.

Available information on this point comes mostly from studies performed in Japan (27), and in indigenous populations of North (28-29) and South America (30). In each of these studies, the serologically detectable HLA-DR antigens could be recognized with serologic patterns that were similar to those obtained with cells of Caucasoid origin. The HLA-D determinants, as defined by using standard homozygous typing cells, were absent (29).

However, new homozygous typing cells were found that identified determinants on Japanese cells, which appeared to take the place of the HLA-D antigens that were missing (Table 1).

Table 1
HLA-B,DR,D HAPLOTYPES IN JAPANESE AND CAUCASOID POPULATIONS

Japanese Haplotypes	Caucasoid Haplotypes
Bw52, DR2, Dw12*	B7, DR2, Dw2
Bw54, DR4, DYT	Bw62, DR4, Dw4
Bw44, DR-, DEn	Bw38, DRw6, Dw6.1

*Dw12 was previously called DHO.

Thus the antigen DHO (now Dw12) associated with DR2 instead of Dw2, DYT took the place of Dw4; and the antigen DEn was found with DR blank in Japanese and appeared to be related to Dw6 in Caucasoids. Along similar lines, Layrisse and co-workers (30) have recently reported on HLA-D determinants which are characteristic of a population of Warao Indians in Venezuela.

The differential expression of HLA-D antigens is generally not complete. For example, Dw12 (DHO) is found also in Caucasoids, associated with DR2, like in the Japanese (31). Thus DR2 associates with Dw2 in some Caucasoid haplotypes and with Dw12 in others.

THE RELATIONSHIP BETWEEN HLA-D AND DR

The close relationship between HLA-D and DR was an impressive finding when the DR antigens were first discovered. It led many investigators to postulate that the DR specificities were simply the serologic expression of the HLA-D antigens. However, it was a common experience, even in Caucasoid populations, that the correspondence was not perfect. There were always some cells that were positive for well defined DR antigens, in which the corresponding HLA-D determinant was absent (28). In our own laboratory, these discordant D/DR typings were observed, both in the unrelated panel and in a number of carefully investigated families, typed with international workshop reagents. The frequency of HLA-D/DR concordant haplotypes in panel members of three ethnic groups, in our laboratory, is shown in Table 2.

Table 2
FREQUENCY OF HLA-D/DR CONCORDANT HAPLOTYPES

Ethnic Group	Number Tested	HLA-D/DR Concordant Number	Percent
N A. Caucasian	75	53	51
Am Indian	30	14	47
Am Black	17	4	24

To further investigate the significance of the apparent lack of agreement between HLA-DR and D typing in panel members, we investigated the effect of concordant and discordant typings on the results obtained in reciprocal MLC. The general conclusion of these investigations was that matching for HLA-DR was predictive for non-reactivity in MLC only if the HLA-D determinants were also matched (32). An example of such an experiment is shown in Table 3.

Table 3
MIXED LYMPHOCYTE CULTURE REACTIONS BETWEEN
INDIVIDUALS HAVING HLA-DR1, DR4

Responders	HLA	Stimulators		
		MS	BS	BF
	D,DR; D,DR	w1,1; w4,4	w1,1; w4,4	w1,1; -,4
		DNV		%
MS	w1,1; w4,4	1	1	67
BS	w1,1; w4,4	5	3	72
BF	w1,1; --,4	63	60	1

Individuals MS, BS and BF had the phenotypes DR1, DR4. The first two were also Dw1 and Dw4, whereas in BF, Dw4 was missing. The results show that MS and BS were mutually non-stimulatory, but showed strong stimulation with BF. A summary of a number of such experiments is shown in Table 4.

Table 4
PREDICTIVE VALUE OF HLA-DR MATCHING FOR MLC
NON-REACTIVITY DEPENDS ON MATCHING FOR HLA-D.

HLA-DR Matched	HLA-D Matched	Number	Results of MLC (DNV)
Yes	Yes	28	15.8 $\pm$ 6.7
Yes	No	33	72.3 $\pm$ 23.7*

* $p < 0.001$

It shows that HLA-D matching had a profound effect on the reciprocal MLC, even when HLA-DR was matched.

The serologic typings of these persons were carefully reviewed,

looking for the possibility of subtle serologic differences. Even when more than 12 sera had been used to define the DR antigens, the HLA-D discrepant cells showed no differences in their serologic reactions. These results only mean, of course, that with presently available reagents, no differences were found. Antibodies may be discovered in the future that may allow one to distinguish between these cells. Similar observations have also been made in certain families. Of particular interest was family No. 14 (Table 5), in which both parents were homozygous for HLA-DR.

Table 5

INHERITANCE OF HLA ANTIGENS IN FAMILY 14

Family Member	HLA Genotypes	HLA Genotypes
Father	A2, B12, Cw5, DRw6, Dw6	A
	A11,B37, Cw6, DRw6, Dw6	B
Mother	Aw24,Bw44,Cw5,DR1, Dw1	C
	Aw24,B14, Cw2,DR1, D-	D
Sib 1		A,C
Sib 2		B,D
Sib 3		B,D
Sib 4		A,C

The father was homozygous DRw6 and also homozygous Dw6. His cells did not stimulate any of the children and functioned as HTCs for Dw6, using unrelated responders. The mother was homozygous DR1. However, a typical Dw1 determinant was found in only one of her haplotypes. This haplotype was inherited by two of the children, whereas the other two children inherited a haplotype having DR1 and D blank. The cells from the mother stimulate all the children and they do not function as homozygous typing cells. Thus, this family illustrates the principle that homzygosity for HLA-DR does not predict homozygosity for HLA-D, and that the outcome of reciprocal stimulation depends on matching for HLA-D, even when HLA-DR is matched.

CELL-MEDIATED CYTOTOXICITY AGAINST HLA-D/DR ANTIGENS EXPRESSED IN MONOCYTES AND B LYMPHOCYTES.

Prior to 1979, it was generally accepted that human cytotoxic lymphocytes did not recognize the HLA-D/DR determinants (33-36). This was somewhat surprising, however, since in the mouse it is well established that cytotoxic cells against the I-region determinants are easily detectable (37). Feighery, in our laboratory (38) found that by using stimulating cells matched to the responder for the HLA-A,B,C antigens and mismatched only for the HLA-D/DR region and by using peripheral blood monocytes as target cells, cell mediated cytotoxicity against HLA-D was easily detectable. The determinants that serve as targets for this type of cytotoxicity, as demonstrated by cold cell inhibition experiments were expressed in monocytes and B lymphocytes but not in T cells. Such cytotoxic cells recognized determinants closely related to the HLA-D/DR antigens in cells from unrelated donors and the cytotoxic reactions could be inhibited by xeno-antibodies against human Ia antigens, alloantisera for the DR specificities, and more recently, with monoclonal antibody against monomorphic determinants of the HLA-DR molecules (39-40). Ball and co-workers (41) recently observed, in a recombinant family, that the target determinants for cytotoxic lymphocytes of this type were encoded to the left of HLA-B.

It is possible also to generate cytotoxic lymphocytes against HLA-D/DR when the stimulating and responding cells are not completely matched for the HLA-A, B, C antigens, but then the target cells have to be carefully selected to avoid presence of HLA-A, B, C antigens against which the effectors might also be active. When this is done it can be shown that the magnitude of chromium release attributable to a single HLA-D/DR antigen is comparable to that obtained when the target is a single HLA-A or B determinant (42).

When the effector cells were sensitized to stimulators carrying concordant HLA-D/DR antigens it was observed that target cells lacking the HLA-D, but having the corresponding HLA-DR, were usually also killed and the amount of chromium released was similar (38). An important question is whether such cytotoxic lymphocytes might also recognize the HLA-D antigen when the DR antigen is not present. Such reagents might then be used for typing for the HLA-D determinants by chromium release cytotoxicity. This would have many advantages over HTC typing. However, at this writing, it is not clear whether the HLA-D determinants can be separately recognized. It seems likely, however, that if properly stimulated, such clones of T cells can be developed. This would be an important new technical development, both for gaining a better understanding of the determinants that are coded by genes of the HLA-D region as well

as offering the possibility of a practical typing procedure for application in clinical studies and for typing for organ transplantation.

ANTIGENS OTHER THAN HLA-D RECOGNIZED BY PRIMED LYMPHOCYTES

In a series of experiments designed to investigate the role of different HLA-D determinants in secondary allogeneic proliferation in man, Sasportes and co-workers (17) found that the HLA-DR determinants seemed to be more closely related to the stimulation of primed cells than HLA-D as defined with homozygous typing cells in primary cultures. Hartzman and co-workers (43) found that HLA-D and DR each were able to cause both primary and secondary stimulation. In their experiments the responder and stimulator pairs were selected in such a way that only one of the two specificities was mismatched and therefore able to stimulate in the primary cultures.

More recently several groups of investigators have provided evidence for the existence of still other HLA linked determinants capable of stimulating in primed lymphocyte tests. Mawas and co-workers (10) studied a family with a recombination between HLA-D/DR and GLO. Primed lymphocytes in this family gave indication of lymphocyte activating determinants separate from those of HLA-D/DR, located centromeric to the HLA-D/DR region. The antigens in this region to the left of D/DR, which they have called Beta, appear to produce weak primary and strong secondary MLR stimulation.

Termijtelen and co-workers (12) used priming between responding cells that were Dw3/DR3 positive and stimulating cells homozygous for Dw3/DR3. THe lymphocytes primed in these experiments appeared to recognize a new determinant. It was different from the HLA-D/DR determinants, was associated with the A1, B8, Dw3, DR3 haplotypes and segregated with HLA in informative families. This antigen was called PL3A. Although it caused mainly stimulation in secondary cultures it also appeared to play a role in the primary MLC (13).

A somewhat similar approach was taken by Shaw and co-workers (11). Extensive priming experiments were performed between subjects who were phenotypically identical, having the antigens A1, A2, B7, B8 and most of them also matched for the antigens Dw2, Dw3, DR2, DR3. By secondary MLC with these primed cells a new series of antigens was defined. It consists of five specificities each defined by at least two primed cells. The antigens were called "SB" for "secondary B cell antigens". These antigens appear to be independent and distinct from the D and DR antigens. They appear to be linked to HLA, in family studies, and in a recombinant were inherited with a portion of the chromosome centromeric to HLA-B. It is of interest that the SB determinants appear to be also targets for cell mediated

cytotoxicity using lymphoblastoid B cells as target cells. However, no serum antibodies that correlated with these specificities were found (44).

Thus the primed lymphocyte test seems suitable for the identification of a number of different lymphocyte activating determinants, some of which are distinct from HLA-D and DR. Recently exchanges between Shaw, Termijtelen and Mawas (Shaw, personal communication) have been perfomed, giving preliminary evidence that PL3A, as well as the Dw3-associated PLT determinant found by Mawas, are similar to the SB1 antigen described by Shaw.

OTHER IA-LIKE ANTIGENS IN HUMAN B LYMPHOCYTES

We have seen that cellular methods have been used to identify some antigens other than the HLA-D/DR specificities which constitute B cell alloantigens that are presently not recognized using B cell typing sera. In addition, we have indications of the existence of serologically identifiable B cell alloantigens distinct from HLA-DR. For example Mann and co-workers (45) performed a series of family studies with B cell alloantisera and obtained evidence of the existence of at least two loci for B cell antigens within the HLA region.

Serologic and immunochemical evidence for the existence of other distinct B cell alloantigens has been obtained by a number of different investigators. Tosi and co-workers (46) found that the antigen DC1 was a serologic specificity distinct from the DR antigens, which appeared to reside in separate molecules, as determined by immunoprecipitation studies.

The supertypic antigens MT1 (identical To DC1), MT2 and MT3 were extensively investigated during the Eighth International Histocompatibility Workshop (47). Duquesnoy and co-workers (48) have evidence of yet another second supertypic system of antigens called MB antigens. The serologic analysis of these specificities poses difficulties because of the complexity of the sera that are used.

In addition to these experiments suggesting heterogeneity of B cell antigens on the basis of the epitopes used to characterize the allogeneic specificities, there appears to exist another kind of heterogeneity recognized by the use of monoclonal antibodies against framework determinants. Lampson and co-workers (49), as well as Quaranta and co-workers (50), reported that monoclonal antibodies, which apparently recognize backbone structures of the human DR antigens and react with B cells and monocytes from most individuals tested, appear to immunoprecipitate distinct molecules. Thus it appears that these monoclonal mouse anti-human DR antibodies are recognizing an isotype-like heterogeneity which is to a certain extent

independent of the allospecificity.

Evidence for additional Ia-like molecules on human B cells is also obtained from the comparison of the reactivity of anti-p23/30 rabbit sera or chicken antibodies, and the monoclonal antibodies against the DR antigens. Particularly revealing in this regard have been studies performed by Nunez and co-workers (51), using sera from kidney transplant recipients. From this work it appears that three kinds of B cell antibodies can be defined: 1. anti-DR antibodies; 2. antibodies against Ia-like antigens other than DR; and 3. antibodies against B cell antigens unrelated to the Ia-like antigens.

The last group of antibodies includes the cold reactive B cell lymphocytotoxins observed in the serum of many kidney transplant recipients and the B cell auto-antibodies which develop in patients with systemic lupus erythematosus. The reactions of these antibodies were not inhibited by pretreatment of the target cells with chicken anti-human Ia.

The anti-DR antibodies were defined by the fact that they were blocked when the B cells were pretreated by monoclonal antibody against monomorphic determinants of human DR antigens. Of particular interest was the second group of antibodies, which was defined by lack of blocking with the monoclonal anti-DR, but complete inhibition by pretreatment of the B cells with chicken anti-Ia. This group probably includes the broadly reactive antibodies MT1 and MT3, as well as other Ia-like specificities which have not yet been defined.

IA-LIKE ANTIGENS OF HUMAN T CELLS AND T CELL SUBSETS

The majority of human T cells circulating in peripheral blood do not have detectable Ia-like antigens. However, a number of investigators have observed that when T cells are activated either under the influence of plant mitogens, such as PHA or Concanavalin A, or by specific antigens, such as heterologous proteins or allogeneic antigens, the DR specificities become detectable on a substantial portion of the T lymphocytes undergoing blastic transformation (52,53). Although binding of donor type antigens to responding T cells is a possibility and has been observed in some experiments, it appears that the majority of the DR antigens expressed in such T cells are of the responder type and are synthesized by the cultured T cells. It has also been reported that in certain patients, who apparently are undergoing antigenic stimulation in vivo, T cells expressing the DR antigens can be found to be circulating in the blood (54).

Recently Gazit and co-workers (55) have reported that human T cell blasts express some HLA-linked antigens which are distinct

from the HLA-DR specificities. These antigens are associated with B_2 microglobulin and are probably not Ia-like.

It is well established from studies in mice that T cells express certain Ia specificities which are different from those expressed in B lymphocytes. These Ia antigens in the mouse have been found to be present in unstimulated T cells.

Van Leeuwen and co-workers (56) have recently reported on alloantisera that reacted with certain T cell subsets. Unstimulated T cells were fractionated on the basis of the presence of Fc receptors for IgG or IgM and antibodies reacting with either the T_G or the T_M type of T lymphocytes were observed. The exact nature of these alloantibodies is not yet known. Many of them were not HLA linked.

In our laboratory Okada has taken a somewhat different approach (57). Normal resting T cells were separated into subsets by the use of monoclonal antibodies against the T cell phenotype markers OKT4 and OKT8. After combination of the antibody with the T cells carrying the marker they were separated by rosetting with ox erythrocyts coated with staphylococcus protein A. Isolated T cell subsets were then utilized for screening alloantisera known to contain antibodies against B cells and monocytes, which had been platelet absorbed to remove HLA-A, B,C, antibodies. In preliminary experiments five sera reacting with the T8 subset and two reacting with the T4 subset were found. One serum, that gave strong reactions with the T8 subset of T lymphocytes, was of particular interest. This serum when used to treat precursor lymphocytes, with rabbit complement, markedly inhibited the development of supressor T cells, but had no substantial effect on the development of cytotoxic T cells. In preliminary experiments the determinant expressed in the T8 subset, recognized by this pregnancy serum, appeared to be inherited in linkage with HLA. Preliminary results suggest that the determinants expressed in the T8 subset may be Ia-like and because of their presence in the precursors of supressor T cells but not of cytotoxic T cells, it is possible that the ZG antigens represent a human equivalent of the mouse I-J determinants.

RECOMBINATION BETWEEN HLA-D AND HLA-DR

Without inbred strains and without recombinants progress in the study of the genetics of the HLA-D region has been slow compared to the work on the homologous region of the mouse. We seem to be quite far from having a complete understanding of the molecular relationships and even further from figuring out the correspondence between genes and cell surface products. In view of the recent evidence that component polypeptide chains of the Ia molecules may be coded in separate genetic areas, and considering the recent evidence of recombinatorial interactions

between alpha and beta chains, it is not easy to make inferences about genetic loci, from the available data.

Several groups of investigators have published observations in families in which they believe a recombination between HLA-D and HLA-DR may have taken place (58-60). Three of these will be briefly reviewed below.

Sachs and co-workers (59) have studied such a family quite extensively. The members were typed for HLA-A, B, C, D, DR, GLO, BF, C2 and C4 and several red cell markers. One member of the family, who should have been Dw7 homozygous, was found to behave as heterozygous. A possible explanation was, that this individual inherited the Dw1 determinant present in the other paternal haplotype through a chromosomal recombination. However, the cells did not behave like the other Dw1 positive cells in the family. Several explanations were offered by the authors to explain the anomalous findings. Their favorite hypothesis is that Dw1 is complex, perhaps composed of two lymphocyte activiting determinants, and that only one of them was inherited by the anomolous family member. Other alternative explantions discussed are the possibility of a mutation in the HLA-D region and the possibility of development of hybrid antigens, products of gene complementation. Another puzzling family was extensively investigated by Reinsmoen and co-workers (58). The family included two siblings, who were HLA-A, B,C and DR identical, but strongly stimulatory in mixed lymphocyte cultures. The hypothesis favored by these authors is that there are two separate, but closely linked loci, HLA-D and HLA-DR, both of which stimulate strongly in primary MLC. Furthermore, the HLA-D region appears to confer the specificity of the early reactions observed in secondary MLC. Other possibilities considered were a spontaneous mutation, detectable by MLC testing but not by serology, and the possibility of stimulation by determinants not coded by genes of the HLA region.

Suciu-Foca and co-workers (60) have studied a family in which one of the children inherited from the father a haplotype with DR7 and Dw3. In reciprocal primary mixed lymphocyte cultures this child was MLC non-stimulatory with a DR3,Dw3, DR5, Dw5 sister, and was said to be MLC-different from the DR7, Dw7, DR5, Dw5 brother. In this family also, the secondary MLCs appeared to be activated by the DR antigens. Thus an interesting discrepancy resulted, since the secondary lymphocyte test did not show the anomolous reactions observed in primary MLC. The authors believe that HLA-D and DR antigens are coded by closely linked, but distinct loci, that HLA-D antigens activate the recognition response in MLC, and DR antigens stimulate the response of primed T cells.

These interesting observations must be interpreted with caution. The D-region may be more complex than revealed by the

discrimination of currently available testing techniques. Study of such families, with unusual typing results, is undoubtedly useful and important. But we must await further developments before we can make definitive interpretations.

DISUSSION

Although striking progress has been made, in just a few years, we are still far from having a complete understanding of the HLA-D region antigens. The parallel development of cellular and serologic typing methods for the products of these genes has given rise to peculiar problems not encountered in the study of the other HLA antigens which are commonly defined by serologic methods only.

The idea that Class I HLA products can be characterized only by serologic tecniques and the Class II products only by cellular methods was initially attractive, but turned out to be incorrect, and has really not been a usefull framework of reference for thinking about these antigens. It is clear that the Class I and Class II type histocompatibility antigens are structurally and anatomically distinct. There are also functional differences. But they are not as crisp and definite as was initially envisioned.

The fact that human cytotoxic lymphocytes can recognize both Class I and Class II specificities is now well established. However, there is recent evidence that the phenotype of the effector cells may be different (42). Not only are the HLA-DR antigens recognized by cytotoxic lymphocytes, but they also appear to be important in stimulation of the proliferation in secondary mixed lymphocyte cultures (17).

It is amazing in retrospect how long it took to resolve the separation between the HLA-A and B loci, and how difficult it was to clarify the separate nature of the mixed lymphocyte culture stimulating determinants and the recognition of HLA-D. One should therefore not be discouraged if progress in understanding the HLA-D/DR antigens seems slow.

The data from the early mixed lymphocyte culture work has clearly shaped our view of the D region, but one must be cautiously skeptical about the picture derived from mixed lymphocyte cultures. Even if one makes a generous allowance for technical difficulties, there is a lack of correspondence between the results of primary and secondary cultures and it takes some work to fit the results obtained by HTC typing to a simple genetic hypothesis. On the other hand it is interesting to note that the results obtained in Rhesus monkeys and chimpanzees are very similar to those obtained in man (61).

Comparison of the HLA-D region with the homologous region of the

mouse has been interesting and stimulating, but must be qualified by the fact that, in spite of the many similarities, there are also some major differences in the structure of the H-2 region of the mouse and the human HLA chromosomal complex. There is recent data indicating that structurally the molecules immunoprecipitated by antibodies against the DR antigens and those produced in mouse strains by immunization across an I-E difference are strikingly similar in their amino acid sequence. However, it has recently been shown that the beta chain of the I-E molecules, which is the polypeptide chain that carries the allogeneic determinants, is coded in the I-A region.

Among the newer techniques that promise to further our understanding of the products and genetics of the HLA-D region one should mention, the investigation of Ia-like antigens expressed only in certain subsets of cells, the analysis of D region products with a multitude of monoclonal antibodies, and the development of T cell clones against HLA-D region products.

Examples of the first approach are the development of reagents which recognize determinants which are uniquely expressed in monocytes or subsets of T lymphocytes. The recently discovered ZG antigens, which are expressed in the precursors of supressor T cells, and also in peripheral blood monocytes, could well represent the human equivalent of the mouse I-J antigens (57).

The use of monoclonal antibodies will undoubtedly revolutionize the definition of the D region products. They have already begun to reveal a complexity that was previously unsuspected (49,50). Further analysis of the D region products using such antibodies promises to be very informative. They should clarify the interactions between different alpha and beta-chains, the possible existence of isotypes, and the different relationships between the allogeneic determinants and the sites recognized by the monoclonal antibodies. If the existence of isotypes is confirmed, it will be of interest to determine their anatomic distribution and expression in different types of cells and the possibility that different isotypes may have different functions.

Both proliferating and cytotoxic T cells can now be raised against HLA-D region products. There is preliminary evidence at least, that both types of T cells can respond to HLA-D and to HLA-DR antigens. Many laboratories are presently devoting efforts to the development of techniques for isolating and expanding clones of T cells to perform an analysis of the HLA-D region antigens.

Cytotoxic assays appear to offer some advantages over proliferative assays. They can be performed in a matter of hours instead of days, and they seem to have better reproducibility.

Immunization in vitro is a viable alternative to in vivo immunization and appears to work in many instances. If the responder and the stimulator are properly selected, usually matched for a number of known determinants and mismatched for the antigens of interest, and if the target cells used express the antigen to be determined, then this approach can be successful. It should be possible to begin to develop batteries of cytotoxic T cells recognizing many different HLA-D specificities. If this works it is not difficult to imagine that assys based on cytotoxicity could replace the tests based on lymphocyte proliferation. Cellular methods are obviously here to stay for some time.

Moreover, it is possible that the development of human hybridomas, secreting specific antibodies, will follow closely, and that eventually it will be possible, also using in vitro planned immunization, to develop monoclonal antibodies against determinants recognized now only by T cells.

CONCLUSION

The human Ia-like antigens determine the collaboration between T cells and accessory cells that present antigen. They appear to play a role in the genetic control of immune responses, similar to that of the homologous systems in other species. They have been found to be markers of genetic predisposition in a variety of human diseases and they appear to play a role in organ transplantation.

Understanding of the HLA-D region and its products has been colored by the peculiar combination of cellular and serologic methods that has been used in their study. Efforts to correlate these various methodologies have sometimes yielded puzzling results.

At the present time we recognize strong lymphocyte activating products detectable in primary MLC and responsible for the HTC typing reactions (D antigens); weaker lymphocyte activating products detectable mainly in secondary MLC; narrow serologic allospecifities (the DR antigens); broad alloantigens (the MT and MB series); and the new specificities recognized by monoclonal antibodies.

There is intense activity in many laboratories for development of new methodologies for HLA-D region studies. A number of new techniques are on the horizon. They include the investigation of determinants expressed uniquely in certain types of cells such as monocytes or subsets of T lymphocytes; the development of monoclonal antibodies; the use of cytotoxic T cells; and the development of T cell clones against the HLA-D region products.

As these methods are perfected much new information will be forthcoming and many of the present difficulties may be resolved.

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OVERVIEW OF THE ASSOCIATIONS OF DISEASES WITH HLA-D/DR ANTIGENS

The accompanying table provides a fairly complete summary of the presently known disease associations with the antigens of the HLA-D region. When available, an estimate of the relative risk is also given. The **relative risk** is a useful numerical expression of the increased risk for development of disease in subjects having the HLA antigen compared to those that do not have it. The relative risk is usually calculated as the cross product of the elements of the two by two tables used for the computation of the chi square.

In many cases representative references were chosen and no attempt was made to provide a complete bibliography. In constructing this table I have made considerable use of the **Third Report of the HLA and Disease Registry** by L.P. Ryder, E. Andersen and A. Svejgaard, published in Copenhagen in 1979. The registry is a comprehensive compilation of HLA and disease associations including all of the known loci and is based on published data as well as private reports made to the registry. More recent data, published after 1978, has been obtained directly from the literature.

A few comments about the disease associations given in the table are worthwhile. Since the table deals only with associations of diseases with HLA-D or DR antigens many diseases which are still incompletely studied and for which associations with HLA-A or B antigens have been suggested do not appear. I have also excluded some reports which look interesting but require clarification by further study.

INFECTIOUS DISEASES

It would be expected that the immune response genes of the HLA-D region would influence resistance or susceptibility to infection. Infections must have provided an important mechanism for natural selection and may in part be the driving force for the remarkable polymorphism of the HLA system. Among the diseases reviewed, there were two conditions, **tuberculoid leprosy** and **ocular histoplasmosis**, that appeared definite enough to include in the table. Several other infectious diseases have been reported to have association with HLA-A or B antigens.

METABOLIC DISORDERS

Autoimmunity against the thyroid has been considered for many years as one of the best examples of autoimmune disease. **Hashimoto's thyroiditis** resembles the animal models of autoimmune thyroiditis in many respects. Thus, it was surprising that **Graves' disease** showed a strong HLA association and Hashimoto's thyroiditis did not. Recently this

paradox has been explained. When the thyroiditis patients were subdivided clinically on the basis of presence or absence of goiter, to subsets were identified. **Hashimoto's thyroiditis with goiter** was associated with DR5. **Atrophic autoimmune thyroiditis** was associated with with DR3. This offers another example of the importance of identifying clinically homogeneous subsets of patients and the effect of HLA associations on disease classification.

It should be mentioned that there is also another form of thyroiditis, **subacute thyroiditis or de Quervain's disease,** which has a rather weak association with DR1 and is strongly associated with Bw35. For this reason, it was omitted from the table. It is however, very interesting because of the role HLA-A,B,C antigens appear to play in recognition of virus-infected cells by cytotoxic T cells.

The antigen DR3 is very rare among Japanese. It is of interest that **Graves' disease** in this population is associated with Dw12, instead of Dw3. Similarly, in **insulin-dependent diabetes** the antigen DYT is found instead of the Caucasian antigen Dw4. It is now well established that the HLA-D/DR associations in diabetes are much stronger than the associations with HLA-A or B. Many investigators have observed that the risk for development of diabetes is the highest in subjects in whom both DR3 and DR4 are present. It has been suggested that in this case the risk may be due to the effect of two interacting genes. An interesting possibility would be the existence of hybrid antigens in the DR3,DR4 heterozygotes which might have a very strong effect on disease predisposition.

ASSOCIATIONS OF DISEASES WITH HLA-D/DR ANTIGENS

Diseases	Race*	HLA-D/DR	Relative Risk	References
Infectious Diseases				
Tuberculoid leprosy	Cau	DR2	8.1	1
Ocular histoplasmosis	Cau	DR2	11.7	2
Metabolic Disorders				
Graves' disease	Cau	Dw3	3.9	3
	Cau	DR3	5.5,4.1	4,5
Graves' disease	Jap	Dw12	4.2	6
Atrophic autoimmune thyroiditis	Cau	DR3	3.4	7
Hashimoto's with goiter	Cau	DR5	3.2,3.7	8,9
Type I diabetes	Cau	DR3	5.7	10
	Cau	DR4	2.8	10
Type I diabetes	Jap	Dw3	28.6	11
	Jap	DYT	4.0	11
Type I diabetes	A Blk	DR3	3.1	12
	A Blk	DR4	4.8,14.2	12,13
Type I diabetes	A Mex	DR4	3.7	13
Type I diabetes	Ashk	DR3	2.9	14
	Ashk	DR4	7.7	14
Type I diabetes	Non Ashk	DR3	15.3	14
		DR4	14.0	14
Idiopathic Addison's disease	Cau	Dw3	6.3	15
Disorders of the Blood				
Pernicious anemia	Cau	DR2	2.0,3.7	16,17
Idiopathic thrombocytopenic purpura	Ashk	DR2	3.3	18
Neurologic Diseases				
Multiple sclerosis	Cau	DR2	4.8	19
Multiple sclerosis	Arab	Dw4	13.1	20
Disorders of Heart and Blood Vessels				
Buerger's disease	Cau	DR2	5.0	21
Takayasu's disease	Jap	Dw12	2.5	22
Takayasu's disease	Cau	DR4	n.a.	23
	Cau	MB3	n.a.	23
Giant cell arteritis	Cau	DR4	8.2	24

ASSOCIATIONS OF DISEASES WITH HLA-D/DR ANTIGENS

Diseases	Race*	HLA-D/DR	Relative Risk	References
Diseases of the Gastrointestinal Tract and Liver				
Celiac disease	Cau	DR3	54.0	25
Ulcerative colitis	Jap	DR2	5.1	26
Chronic active hepatitis	Cau	DR3	6.6	27
Diseases of the Kidney				
Goodpasture's syndrome	Cau	DR2	13.1	28
Membranous glomerulonephritis	Cau	DR3	10.7	29
IgA nephropathy	Cau	DR4	3.9	30,31
Minimal change nephrotic syndrome	Cau	DR7	4.5	32,33
Diseases of the Skin				
Dermatitis herpetiformis	Cau	DR3	56.4	34,35
Pemphigus vulgaris	Jews	DR4	14.4	36,37
Herpes gestationis	Cau	DR3/DR4	23.5	38
Rheumatic diseases and Disorders of the Locomotor system				
Rheumatoid arthritis	Cau	DR4	6.0	39
Rheumatoid arthritis	Jap	DR4	2.8	40
	Jap	DYT	4.0	40
Rheumatoid arthritis	A Blk	DR4	5.0	41
Rheumatoid arthritis	A Ind	DR4	13.4	42
Juvenile arthritis, seronegative	Cau	DR5	7.0,6.3	43,44
	Cau	DRw8	5.0,4.25	43,45
Juvenile arthritis systemic	Cau	DR2	3.6	46
Systemic lupus erythematosus	Cau	DR3	3.0,5.7	47,48,49
	Cau	DR2	3.6	47
Subacute cutaneous lupus erythematosus	Cau	DR3	11.8	50
Dermatomyosistis	Cau	DR3	3.9	51
Scleroderma	Cau	DR5	5.0	52
CREST syndrome	Cau	DR3	6.2	53
Sjogren's syndrome, primary	Cau	DR3	3.9	54

ASSOCIATIONS OF DISEASES WITH HLA-D/DR ANTIGENS

Diseases	Race*	HLA-D/DR	Relative Risk	References
Sjogren's with arthritis	Cau	DR4	4.7	54,55

* abbreviations for races: Cau, Caucasoid, Jap, Japanese; A Blk, American Black; A Mex, Mexican American; Ashk, Ashkenazi; Non Ashk, Non-Ashkenazi Jews; A Ind, American Indian.

n.a. = not available

DISORDERS OF THE BLOOD

Two conditions have been included in this group. They are interesting because of their association with DR2. The autoimmune nature of **pernicious anemia** and it's relationship to the so called organ specific group of autoimmune conditions is well established. One would therefore expect this disease to be associated with DR3, yet in two separate studies an association with DR2 was found. The association with **idiopathic thrombocytopenic purpura** comes from a study performed in New York in a population composed predominantly, although not exclusively of Ashkenazi Jews. It will be interesting to see whether other studies confirm this association.

NEUROLOGIC DISEASES

The association of **multiple sclerosis** with Dw2 was the first D locus association discovered. Studies in several other neurologic diseases are still preliminary and require confirmation.

DISORDERS OF THE HEART AND BLOOD VESSELS

Three conditions have been included in this group. **Buerger's disease** in Caucasoid patients have been found to be associated with DR2. **Takayasu's arteritis**, also known as the aortic arch syndrome, has been studied in Japan and in North America. Somewhat surprisingly, the associations do not seem to correspond. The Caucasian equivalant of Dw12 is Dw2, but instead, DR4 has been found in a small group of Caucasoid patients. Further studies will be needed to confirm these results. **Giant cell arteritis** in Caucasoids has been found to be associated with DR4.

DISEASES OF THE GASTROINTESTINAL TRACT AND LIVER

The association of **celiac disease** or gluten sensitive enthropathy with DR3 is a very strong one. It is related also to the association of dermatitis herpetiformis with the same HLA-DR antigen. **Chronic active hepatitis** of the autoimmune type has been reported to be also associated with DR3.

DISEASES OF THE KIDNEY

Diseases of the kidney have important HLA-DR associations. They include **Goodpasture's syndrome** with DR2, idiopathic **membranous glomerulonephritis** with DR3, **IgA nephropthy** with DR4, and minimal change, steroid responsive, **nephrotic syndrome** with DR7.

DISEASES OF THE SKIN

A number of dermatologic conditions have important HLA associations. Three diseases with definite associations with DR were selected for the table. **Dermatitis herpetiformis** has already been mentioned above. **Pemphigus vulgaris** in Israel, appears to be associated with DR4. An unusual skin disease of pregnancy, called **herpes gestationis**, has been found to have a very strong association with the simultaneous presence of DR3 and DR4. This is a likely candidate for hybrid antigens.

RHEUMATIC DISEASES AND DISORDERS OF THE LOCOMOTOR SYSTEM

This section is quite large. Many of the rheumatologic disorders are immunologically mediated and have important associations with HLA-D. The association of **rheumatoid arthritis**, developing in adults, with Dw4 and DR4 has already been discussed above. Seropositive polyarthritis developing in children is a relatively uncommon condition, but it also is associated with DR4. The seronegative arthritis of children has recently been found to be associated with DR5 and DRw8. An interesting association of DR2 with **juvenile arthritis** of **systemic onset** has been recently observed in an analysis of the Dallas material. **Systemic lupus erythematosus** is a heterogeneous condition and so the associations are not very strong. **Subacute cutaneous lupus erythematosus** is a distinct subset which has been described in detail by Sontheimer and coworkers (50) and which has a very strong association with DR3. The reports on dermatomysistis **scleroderma** and **CREST syndrome** are still preliminary and need confirmation. It is rather interesting that **Sjogren's syndrome** without arthritis has a very strong association with DR3, while Sjogren's syndrome appearing in patients with arthritis shows no increase of this antigen and instead shows the increased frequency of DR4 commonly found in patients with rheumatoid arthritis.

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