Heberden Oration

HLA as regulator

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When Derrick Brewerton invited me to give the annual Heberden Oration he wrote that after the initial excitement about the associations of HLA and disease the impetus of the research was in danger of being lost and could I come over and tell 'what to do about it.' It is obvious first that this is the kind of invitation you simply cannot turn down and secondly that it is an impossible task to fulfil. Derrick told me also that I could 'speculate a bit,' and as he is your president, I shall take the opportunity.

I would like to divide my lecture into three parts. In the first I shall briefly review recent developments on the genetics of HLA; these are hard facts. In the second I shall present to you data which indicate that HLA class I molecules might have an important role not only in immunology but also in endocrine functions, which are new facts, and their interpretation is not yet definitive. In the third and last part I shall discuss some findings in leprosy which might be useful in the study of the pathogenesis of rheumatoid arthritis (RA) which is almost all speculation. In the two last topics HLA acts as a regulator. I realise that I shall discuss mainly topics outside the field of rheumatology, but Derrick told me to approach my topic with breadth of vision.

Genetics of HLA

Phase 1 is thus the genetics of HLA. Fig. 1 brings you up to date with the present situation. In the last few years the HLA region has at least doubled in size and 'grown' perhaps even much more. The broken line indicates what we today consider might encompass the HLA supergene and the closed rectangle was what it was like until a few years ago. Then only the ABC and D/DR loci were known, coding respectively for the class I antigens, which are expressed on all nucleated cells, and the class II

antigens, which express themselves on cells involved in the immune response like macrophages, activated T cells, and B cells. I will not discuss the class III genes coding for complement factors today. In recent years at least two class II loci have been added to the list; one is the DC or MB locus, which is certainly located to the left of HLA-B but of which the exact position relative to HLA-DR is uncertain. It codes for class II antigens. And to the left of D/DR, about 2 centimorgan away from it, is the locus recognised by Termijtelen, Mawas, and Shaw, the secondary B locus or SB. Thus the doubling of the size of the HLA supergene.

On the top line the position of another locus is indicated, the TC-A locus. This locus represents a diallelic system coding for a product present on part of the T-gamma cells.

Recent family studies performed by Van Leeuwen, who identified this locus, indicate that it is on chromosome 6 and about 15 cM away from HLA-A or even less. We can thus recognise the products of three more loci than those officially recognised so far (the official ones are HLA-ABC D/DR).

Recently Termijtelen, using cellular typing techniques, has found evidence for yet another locus, the LB-Q locus. The number of genes identified at the chromosomal level is even greater than the number of allelic series identified at the membrane level, and this is true both for class I and class II. By typing by serology we see thus only a fraction of the complexity at the DNA level. There appear to be more than 30 genes coding for the heavy chains of class I and up to 5 α and 7 β genes coding for class II. The class II molecules consist of two polypeptide chains each of which is coded for by one α and one β chain. The β genes carry the allelic specificity with the exception of DC, which is polymorphic as well. Of course not all of these need to be expressed at the cell surface. With protein chemistry techniques up to four class I and six or even seven class II molecules coded for by different genes have been

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Fig. 1 The expanding HLA supergene. □=Class I molecules. ■=Class II molecules. Class III molecules. ---=sequence order unknown.

recognised. As Fig. 1 shows, by typing either by cellular or serological techniques so far only four to three allelic series have been recognised for each of the two classes of products. Before leaving genetics there are two further points which I would like to emphasise. The first is that the individual class I and II molecules carry not one epitope or immunodeterminant which can be recognised by an antibody or a T cell but a large number of epitopes. The epitopes which can be found on one class I molecule, can be detected by alloimmune antibodies, by monoclonal antibodies, or cytotoxic T lymphocytes.

We should realise that each and any of these molecules has first been detected by a single reagent reacting with a single determinant on a given molecule. When other reagents broaden our vision it turns out that there is not one but several similar and different epitopes on that molecule until a picture develops with the simplicity and complexity of a drawing by Escher (Fig. 2), that is, an artist's view of what might be a class I antigen: each bird, each fish an epitope an immunodeterminant with its own function. This is a very important point. If, for instance, we fail to identify the B27 epitope, which occurs most frequently in ankylosing spondylitis (AS) patients with the available monoclonal antibodies or CTLs, this does not mean that such an epitope does not exist. It might simply mean that we

have not used, or do not yet have available, the right reagent. Serology and especially CTLs will remain most helpful, but the final answer will come from the combined knowledge gained with such reagents plus that from DNA technology and protein chemistry. It is now technically feasible to synthesise modified molecules and insert them in artificial vesicles to map more precisely the location and function of the different epitopes.

The biochemical studies have also unearthed another very important finding and that is the homology of the membrane-adjacent part of the class I and II molecules, β -2-microglobulin and the constant regions of the Ig heavy and light chain.

The chemistry of class I and class II molecules has been worked out to a large extent. The biochemical evidence indicates that immunoglobulin genes might have arisen at least in part from the same primordial gene as the class I and class II genes of HLA. The following are the most relevant findings. First, the amino acid sequence of heavy chain domains of IgG and IgM is significantly homologous to that of the membrane adjacent section of the class I and class II antigens. Secondly, it is quite likely that the three-dimensional structure of class I and II antigens (based on their amino acid sequence) will be similar to that of the Fab part of immunoglobulin. Thirdly, and finally, the variable regions of the class I and II

Fig. 2 'Lucht en water' by M. C. Escher. (From The Graphic Work of M. C. Escher.)

antigens (which are thus the epitopes, or that part which will react with the antibodies used for HLA typing) are located on the same part of the molecule as the various regions where the immunoglobulins are located. Now if the HLA molecules in their amino acid sequence and three-dimensional structure resemble immunoglobulins, then a logical question is: will they also *function* as immunoglobu-

lins, that is, will they be able to interact with foreign molecules? We think they do and I will discuss this in the second part.

To summarise the first part: (1) far more allelic series than the well known HLA-ABC D/DR exist; (2) a single class I or II molecule carries many different epitopes; (3) class I and II molecules in part resemble immunoglobulins.

Fig. 3 Arrangement of the class I and II antigen domains, which may adopt a similar overall structure. (Larhammar et al. 1983).

Class I molecules

We come now to the second part of my talk—the class I molecules. The message here is that our group has been working on the hypothesis that the class I molecules, and probably also the class II molecules, are similar not only structurally to Ig but also functionally and that they can be best compared with primitive cell membrane bound immunoglobulins, that is, molecules which can interact with and thus bind a variety of autologous or exogenous molecules. This complex can be endocytosed. Depending on the material (and probably route of endocytosis) it can either be broken down or form an essential nutrient for the cell. If the endocytosis fails, it is reprocessed in another way (enzyme breakdown?) and can then activate the immune apparatus.

The best evidence for the endocytosis–transport function—comes from experiments of Per Peterson's group. They provided biochemical evidence that the heavy chain of class I can combine with a viral protein, that this complex is endocytosed and passes through the vesicular bodies to the lysosomes, where the viral protein is broken down.

Fig. 3 was drawn by Peterson from biochemical data; the actual order of events might be more complex than is suggested by this drawing. Frans Claas in our group has mainly studied what happens at the level of the cell membrane. The technique he used is a simple blocking test in which cells, mainly but not exclusively lymphocytes, are incubated with

the molecules under study (drugs, antigen, etc.) and next tested with antibodies which normally react with HLA and non-HLA antigen present on the cells used. If blocking occurs, it is assumed that the molecule in question has interacted with the epitope recognised by the antibody or another very close epitope (steric hindrance).

To study antigen-binding by class I molecules model studies have been performed with penicillin (Table 1). If you take lymphocytes from an individual which carries the antigens HLA-A2, and HLA-B5, HLA antibodies together with complement will be able to kill all the cells: we have 100% lysis in both cases. If you first incubate these cells with penicillin and then 15 min later add the HLA

Table 1 Affinity of penicillin for allotypic determinants on class I as compared to that of HLA antibodies

Sequence of incubation	% lymph. dead HLA antibodies	
	α -A2	β -B5
A. 1. Penicillin+cells (15 min) 2. HLA antibodies	100	10
B. 1. Penicillin, HLA antibodies + cells simult	100	10
C. 1. HLA antibodies+cells (15 min) 2. Penicillin	100	80
Control: HLA antibodies+cells (30 min) without penicillin (Claas 1980)	100	100

antibodies and complement, the interaction with the anti-A2 antibody is still possible, that is, there is still 100% kill. However, the anti-B5 antibodies have been inhibited almost completely, that is, there is almost no kill. If you reverse the order, so that first the antibody is put in and 15 minutes later the penicillin, there is hardly any inhibition. We interpret the data as being caused by simple steric hindrance. The HLA molecules carry epitopes which correspond with the A2 and B5 specificities. The A2 specificity or epitope is unable to interact with penicillin while the B5 epitope can do so. When an interaction between penicillin and the epitope has taken place, there is, so to speak, no room for the antibody to interact with the epitope and the interaction thus cannot take place. This is only one example of several that have been found, and we think this might well be a rather general phenomenon. Through this and other studies we are convinced that class I molecules can interact with a large number of foreign exogenous molecules.

In the last year or so it has become clear that not only exogenous molecules can interact with class I antigens but that these class I molecules form an integral part of hormone receptors as well and can thus interact with autologous molecules, the hormones. Simonsen is one of the people who has been very much involved in this new area of HLA. He even introduced a new term for it, the compound receptor, which he defines as 'a molecular complex which attains its final, biologically active structure by rearrangement and assembly in the membrane, of structural subunits.' One of these subunits is the HLA class I molecule. The evidence obtained together with Olsson and Honsik is shown in Table 2.

At more or less the same time as Simonsen and his colleagues were performing their studies on insulin Claas investigated (for reasons which will become

clear in a moment) whether not only penicillin but also gamma endorphins could interact with class I molecules. Gamma endorphins are neuropeptides which are formed in the brain and play an important role in maintaining psychic equilibrium. They are simple and well defined peptides. The technique he used is the same simple blocking test as he used to study the interaction of penicillin and class I molecules. Claas could indeed show that gamma endorphins can (partially) block the interaction of HLA antibodies and some HLA antigens, but not others. There existed a clear polymorphism. Now why was Claas interested in studying gamma endorphins?

The answer is simple. In our studies on HLA and disease one of the diseases studied was schizophrenia. Some schizophrenic patients will improve remarkably and significantly if treated with gamma endorphins. As they had been typed for HLA, it was easy to check whether improvement after gamma endorphin treatment was related to the HLA antigens present in the patient. A remarkable polymorphism was found. For instance, patients who were HLA-B15 positive showed the best response, while patients who carried the B16 or B17 antigens showed no improvement at all. It is of interest that the antigens which correlate with the best therapeutic responses are the same as those which were blocked most effectively by gamma endorphins (Fig. 4). There even exists a significant association between the psychological improvement (as measured by the BPRS (brief psychiatric response score)) and the summarised inhibition for the different class I antigen in individual patients. This not only opens the way to predicting which patient might benefit from gamma endorphin therapy, but opens up a whole new field of HLA functional studies and might, if confirmed, have a major impact on our thinking on the biology of HLA.

There are of course also a number of difficulties with these experiments. Although functional tests have provided evidence for the presence of gamma endorphin receptors on lymphocytes, class I antigens have been hard to find in brain tissue. But these studies were performed a long time ago and were done by absorption and not histology. They thus do not exclude the possibility that some brain cells, perhaps only a few, carry class I antigens. At the same time as the interaction of insulin and gamma endorphin with class I molecules was studied in Copenhagen and in Leiden some French workers at INSERM using precipitation techniques, and Israeli workers at the Weizmann Institute studying insulin and epidermal growth factor, also came to the conclusion that these hormones can interact with class I molecules and that the class I molecules form

Table 2 *The insulin compound receptor*

<i>Human neoplastic cell lines</i>		
Binding of insulin after capping with:		
anti- β_2 m		neg
monoclonal anti-class I		neg
anti-receptor		neg
monoclonal anti-class II		pos
Binding of monoclonal anti-class I heavy chain after preincubation with:		
insulin	50%	inhibition
glucagon	50%	inhibition
somatostatin	50%	inhibition
ACTH	0%	inhibition
vasopressin	0%	inhibition
(Simonsen and Olsson 1983)

Fig. 4 Correlation ($r=0.67$, $p<0.005$) between improvement upon therapy with γ -endorphins shown by schizophrenic patients carrying a given HLA class I antigen (as compared with patients lacking the antigen) and inhibition (%) of that HLA antigen by γ -endorphin. (Claas *et al.* 1983).

an integral part of the corresponding hormone receptors. Thus four different teams using different approaches all came to the conclusion that class I molecules form an integral part of hormone receptors.

I would like to emphasise that the correlation which has been found between HLA polymorphism and blocking capacity in vitro versus therapeutic effect in vivo is so far a unique aspect of the gamma endorphin study. It suggests that the HLA polymorphism influences the effectiveness of the gamma endorphin receptor. These data are completely contrary to present-day dogma. A lot more work has to be done before the sceptics will be convinced. Thus again, these are new findings, which need further confirmation and analysis. If true they may well have a major impact on our thinking in many fields and certainly also in studies on HLA and disease.

To what extent these findings might be relevant in rheumatology is hard to say. Geczy's findings on klebsiella, which are so fascinating and so difficult to reproduce, of course come to our mind. Another interesting aspect of hormone receptors is that they are differentiation antigens in the sense that they occur on some cells but not on others. I must confess that I do not know of a receptor with a predilection

for the sacroiliac joints. How relevant this is for rheumatology thus remains to be seen.

Leprosy

This brings me to the third and final topic which I would like to discuss: leprosy and why it might be of interest to rheumatologists. I will restrict myself mainly to the tuberculoid form of leprosy, in which cellular immunity is present but clearly diminished.

Segregation studies in families in which two or more siblings suffer from leprosy have shown that affected siblings more often share the same paternal haplotypes than expected. This is true both for tuberculoid and, as recently documented, for lepromatous leprosy. Note, however, that there exists no significant deviation in the healthy sibs (Table 3). This indicates that it is not the susceptibility for leprosy that is determined by HLA but the type of leprosy (lepromatous or tuberculoid). If it were otherwise one would expect to find that the healthy sibs also shared a parental haplotype more often than expected, namely, the one which confers resistance. This is not the case. Although the frequency of HLA-DR3 in a population study is no different in patients suffering from leprosy as compared with healthy controls, DR3 is very frequent in tuberculoid leprosy patients and virtually absent in lepromatous ones (Table 4). It thus predisposes to tuberculoid leprosy but protects against lepromatous leprosy. This exemplifies the point I just made: HLA determines not susceptibility for but the type of leprosy. And this is very reminiscent of the situation in RA.

Table 3 Children suffering from TT or BL/LL leprosy share parental HLA haplotypes more frequently than expected

Children	Obs.	Exp.	χ^2	<i>p</i>
TT leprosy	188	139.6	19.36	
5×10^{-6}				
BL/LL leprosy	49	34.5	6.48	0.005
Healthy	42	44	0.06	NS

(van Eden *et al.* 1983)

Table 4 HLA-DR3 and leprosy (Surinam creoles)

	DR3	
	Pos.	Neg.
Controls	23	63
Leprosy	21	48
tuberculoid	16	13
lepromatous	1	22

De Jongh has recently, together with Valkenberg, performed a prospective epidemiological study of HLA in RA in an open Dutch population of about 6½ thousand inhabitants who were investigated for the presence of RA and five years later reinvestigated. Although DR4 was increased in this population in the RA patients with radiological abnormalities and/or rheumatoid factor, the data did not attain significance and were much lower than found in clinical patients and in patients suffering from Felty's syndrome, as has also been observed by Festeinstein and others. The message here is too that DR4 does not predispose to susceptibility to the disease but to its severity (Table 5).

Back again to leprosy. The relation with DR3 was of course a challenge to search for an in-vitro correlate. To this end purified T cells from HLA-DR3 positive heterozygous leprosy patients were incubated with HLA-DR3 homozygous monocytes obtained from healthy donors together with *Mycobacterium leprae* antigens. As a control monocytes from donors homozygous for the other HLA-DR antigen present on the T lymphocyte of the patient were used. We expected that proliferation as measured by ³H thymidine would be lower with DR3 than the non-DR3 monocytes. With one exception HLA-DR3 homozygous monocytes indeed presented MLW-1, the lepra antigen, less efficiently than HLA-DR-non-3 homozygous monocytes (Fig. 5). This could correlate with the clinical situation: there is a cellular immune response in DR3 positive patients (precluding the development of lepromatous leprosy where it is absent), but it is suboptimal, leading to prolonged disease. Although our data are in agreement with such a hypothesis, they certainly do not prove it. On an average the proliferation after presentation by homozygous 3/3 antigens was as low as in the combination in which monocytes and T cells did not share an HLA-DR antigen at all. For the topic we are discussing it is of special interest that the stimulation by DR3/3 monocytes was equal to that of DR non-3/non-3 monocytes if PPD was used as an antigen. The message from these data is that in leprosy two things are known: the causative agent, *M. leprae*, and a gene DR3 which influences not the susceptibility to the disease but its clinical severity.

Table 5 Frequency of HLA-DR4 and RA in an epidemiological study

RA total	n= 53	DR4 32%
RA+RAD/RF	n= 16	DR4 38%
Controls	n=201	DR4 24%

(de Jongh et al. 1983)

Fig. 5 Relative regression coefficients of responses of T cells obtained from HLA-DR3 positive heterozygous leprosy patients, cocultured with either DR3 or DR-non-3 homozygous allogeneic DR-compatible monocytes as antigen presenting cells for MLW-1 antigen. *p*-value was calculated from Wilcoxon's test for paired samples (2-sided). (Van Eden et al. 1983).

We have thus an in-vitro test which correlates with these findings. Or to put it in another way; if we do not know whether *M. leprae* or *M. tuberculosis* predisposes to leprosy, this test could be used as an indicator that *M. leprae* might be the causative agent.

The same protocol has been used by Thorsby in another disease. Gluten enteropathy occurs almost exclusively in DR3 or DR7 positive individuals. When T lymphocytes were used from donors who were heterozygous for DR and DR3 positive, and these were stimulated by monocytes from donors who shared one DR antigen with the T cell donor and were either DR3 positive or positive for the other antigen, a striking difference in proliferation was seen. The proliferation of gluten reactive T cells was lower with DR3 positive monocytes than with the DR non-3 positive monocytes. However, when varicella zoster or PPD was used, both DR3 and the DR non-3 antigen were equally effective in stimulating the proliferation.

Again, if we did not know that gluten is involved in the pathogenesis of gluten enteropathy and wondered whether varicella zoster or purified protein derivative (PPD) might be involved, this test might have given us a clue.

Now why is this of potential interest to rheumatologists? What causes RA is of course not known,

and there is even debate whether a virus or microbial agent plays a role at all, though epidemiological studies especially from Africa might argue for this. A number of possible causative agents have been proposed, including Epstein-Barr virus.

The analogy between leprosy and RA is then as follows: (1) in leprosy a microbial causative agent is known, in RA it is suspected, at least by some of you; (2) in both diseases (I am speaking now of tuberculoid leprosy not of course of the lepromatous form) the micro-organisms is either not found in the patient or found only with great difficulty; (3) in leprosy we have an in-vitro test in which *M. leprae* behaves differently from *M. tuberculosis* and other bacteria and viruses; and (4) it might then be possible to use a similar protocol to screen for possible causative organisms in RA. We are at least planning to give it a try.

This brings me to the end of my talk. I have been asked to renew impetus and I do know whether I have been successful in that. But one thing I do know: either you think that part of what I told you might be true and that might stimulate your working along the lines I discussed or you think it is all rubbish and then you will be forced to think of

something better to do. So in either case activity will ensue, and that is all that matters.

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