Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

Human Histocompatibility Antigens*

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A. Introduction

Numerous chapters in this volume testify to the importance of molecular biology the analysis of problems relating to human cancer. Most recently, it was shown that human (onco)genes carrying single nucleotide substitutions may confer the transformed phenotype rather than encode a presumably normal – albeit unknown – cellular function [1]. With reference to the study of leukemia, recent advances have demonstrated the existence of human leukemia viruses (this volume), and undoubtedly their molecular biology will equally be of great interest for our understanding of these tumors.

It is usually assumed that our first line of defense against tumors is the immune system. In the concept of immune surveillance, cytotoxic T-lymphocytes eliminate malignantly transformed cells. Only when such cells escape the immune system – for whatever reason – may a tumor arise. It has been known for some time that T-lymphocytes in their interactions with target cells, either for cooperative or destructive purposes, are guided by the products of the major histocompatibility complex (MHC) [2]. A thorough understanding of the structure and biologic role of this genetic region should contribute to our knowledge of how "foreign" cells are eliminated – and hence, how tumors may be prevented from establishing themselves.

* This research was supported by the DFG through Sonderforschungsbereich 74

B. The Major Histocompatibility Complex

Originally discovered through skin grafting in mice and through the characterization of leukocyte-agglutinating antibodies in man, the major histocompatibility complex (MHC) has become of central importance in immunology. In humans, the MHC is located on the short arm of chromosome 6 and spans a region of approximately 1.5 centimorgans. The MHC encodes several kinds of membrane glycoproteins, most conveniently referred to as class I and class II antigens [3]. Class I antigens consist of an MHC-encoded membrane glycoprotein chain of 44,000–48,000 daltons in association with a β_2-microglobulin, which is not encoded by the MHC [4]. Class II antigens are built up as a two-chain structure as well, but both subunits are anchored to the membrane and – at least in the mouse – both subunits are encoded by the MHC [5].

The single most striking feature of the MHC is the polymorphism of its products. Both in man and in the mouse, some 30–50 different alleles are likely to exist at the different class I loci (*H-2K, D,* and *L* in the mouse, *HLA-A, -B,* and *-C* in man, to name but the most familiar ones) and a similar situation probably applies to class II antigens [6]. The molecular nature of this polymorphism has been the object of intense study, and the picture that emerges points to the importance of amino acid substitutions in the polymorphic chains in generating the different alleles. Studies of mouse H-2 mutants have been particularly informative in this respect [7]. So far, the

possibility that the MHC encodes carbohydrate rather than protein determinants has received attention in passing. The difficulties of structural analysis of complex carbohydrate structures (e.g., glycolipids) is one of the reasons this area has remained relatively underdeveloped.

The polymorphism of the MHC is thought to be of functional significance. The associations with disease in man constitute a clear argument: Certain diseases are found with elevated frequencies in individuals carrying particular alleles or a constitution of alleles (haplotypes) at the *HLA* loci [6]. Quite often these diseases are, or are thought to be, associated with the immune system.

Thus, a thorough analysis of the polymorphism of the MHC and its functional ramifications should be carried out. Peptide-mapping and protein-sequencing studies have been the methods of choice in the past, but more recently recombinant DNA methodology has gained a foothold. At present, this area is undergoing explosive development, and at the time of writing cDNA clones for virtually every class of MHC-encoded protein have been obtained. Using these cDNA clones, large sections of the murine MHC have been cloned, and in the near future we shall see the construction of a complete physical map of the MHC [8, 9].

C. Class I Genes

By classical serology and immunogenetics, three class I loci have been defined in man so far – *HLA-A, -B,* and *-C* [6]. However, analysis of total human genomic DNA by Southern blotting has revealed the presence of at least 20 class-I-related sequences [10]. In the mouse, the same situation is found and has in fact been studied with higher resolution – 36 class I genes have been cloned from the BALB/c genome [11]. The number of class I antigens identified by genetics and serology is far smaller, however [12]. How can we explain this discrepancy?

First, it is possible that a large number of class I genes are expressed only in specialized tissue or cell types, making their detection by immunogenetic and serologic means difficult. Secondly, it is possible that we are dealing with a relatively large number of pseudogenes. Experiments in the murine system suggest that the latter possibility is at least in part correct: A number of class I pseudogenes have been identified [13, 14].

To address this question, we decided to investigate a parameter thought to relate directly to gene activity. It has been demonstrated in a number of systems that the state of methylation of eukaryotic DNA correlates with gene activity [15]. Hypermethylation of the CpG dinucleotide is generally found to be associated with a lack of gene activity, whereas hypomethylation at these sites is found in actively transcribed genes [15]. The state of methylation can be rapidly assessed by using the isoschizomeric pairs of enzymes Hpa II and Msp I, the latter being insensitive to the presence of methyl-cytosine in the CpG dinucleotide, whereas Hpa II is sensitive.

When human DNA was digested with EcoRI, followed by digestion with Msp I or Hpa II, a clear-cut result was obtained (Fig. 1), namely that the vast majority of human class I sequences, as detected by Southern blotting using an HLA cDNA clone as a probe, were hypermethylated. In the light of current opinion, this would imply that such genes are in an inactive state. Provision has to be made, of course, for the fact that no fine mapping can be carried out due to the presence of a large number of homologous sequences. When the pattern of methylation was examined in DNAs obtained from different tissues of the same individual, no differences were apparent. This would suggest that the degree of methylation of human class I sequences, as detected by these methods, does not vary much from one tissue to another. Secondly, it is apparent that few differences in banding patterns are observed between the different individuals. Although we are dealing with a serologically extremely polymorphic system, this polymorphism is not readily apparent at the level of the DNA that encodes these antigens. A larger survey of enzymes, with different recognition sequences, indeed shows that such polymorphisms can be visualized [10, 16], but the efforts involved are too great when compared with the relative ease of serologic

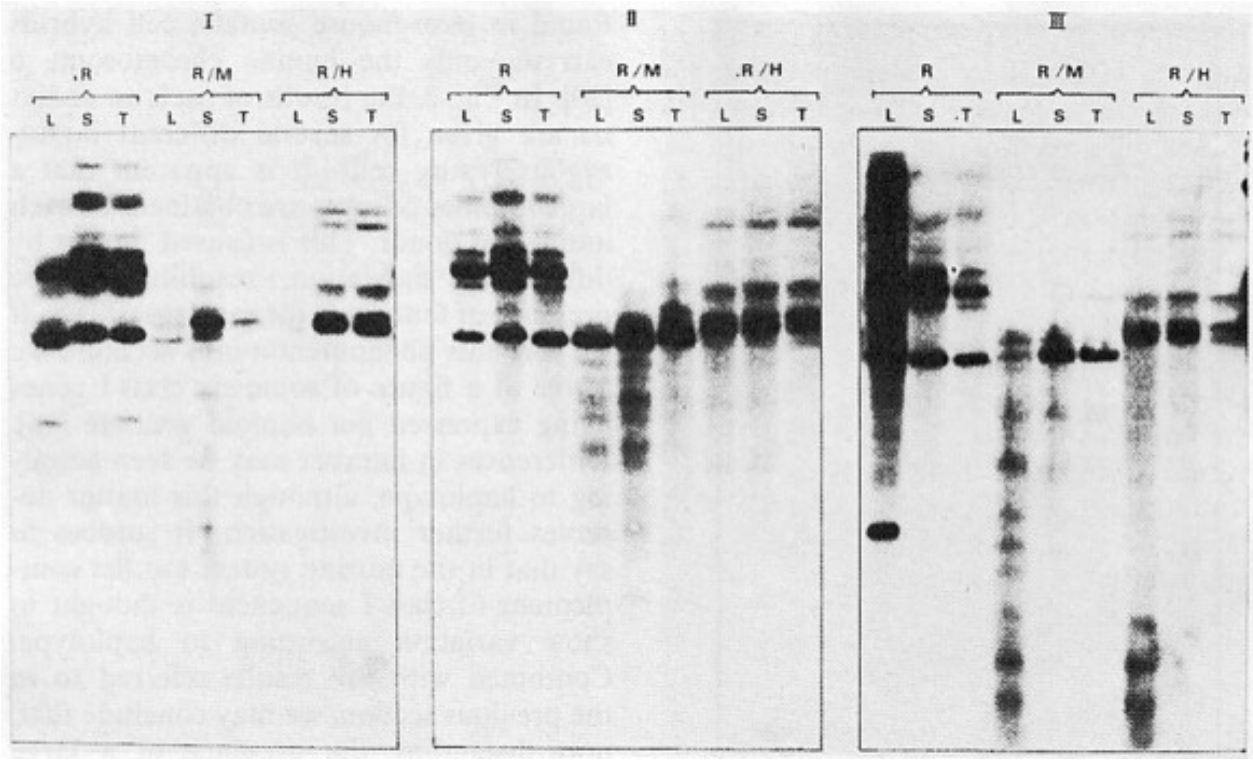

Fig. 1. Analysis by Southern blotting of human class I sequences. DNA from three different individuals was digested with Eco RI, and subsequently with Msp I or Hpa II, as indicated in the *figure.* After agarose gel electrophoresis of the DNA fragments on a 0.8% gel, DNA was transferred to a nitrocellulose filter and probed with nick-translated HLA cDNA clone. Note the apparent lack of polymorphism between the different tissues obtained from a single individual (liver, *L*; spleen, *S*; thymus, *T*) and between different individuals. Note also that most hybridizing sequences are not cut by Hpa II, indicating they are methylated

typing or the resolution of protein-chemical analysis.

D. Class I Antigens

A slightly different approach would be to ask how many gene products can actually be detected. It should be borne in mind that usually alleles at the *HLA* loci are defined by alloantibodies, in which single specifities and their cross-reactive specificities are recognized using the best typing sera available. The advent of monoclonal antibodies has made it possible, however, to obtain xenogeneic antibodies of high titer that react with all HLA specifities examined to date. For example, the mouse monoclonal antibody W6/32 reacts with peripheral blood lymphocytes from humans to a similar extent regardless of the donor's HLA type [16]. Similarly, monoclonal anti-β_2-microglobulin antibodies are available [17] that should react with all class I antigens, as defined by their association with β_2-microglobulin. The use of such antibodies, either alone or in combination, should allow the estimation of the number of class I antigens present in a certain cell type, for example, in mitogenically activated lymphocytes. To examine this question, peripheral blood lymphocytes are labeled with ^{35}S-methionine; class I antigens can then be isolated from detergent extracts prepared from such cells after detergent solubilization. Display of the isolated class I antigens by a separating technique with high resolving power then enables direct counting of the number of different genes expressed [18, 19]. For humans, the lack of inbred strains – one of the few advantages of analyzing the murine system – can be compensated for by the use of cells from homozygous donors, usually the offspring of consanguinous marriages. In such so-called homozygous typing cells, the contributions of a single haplotype can be assessed [18]. At this point, it should be mentioned that all class I sequences that hybridize with HLA cDNA clones can be

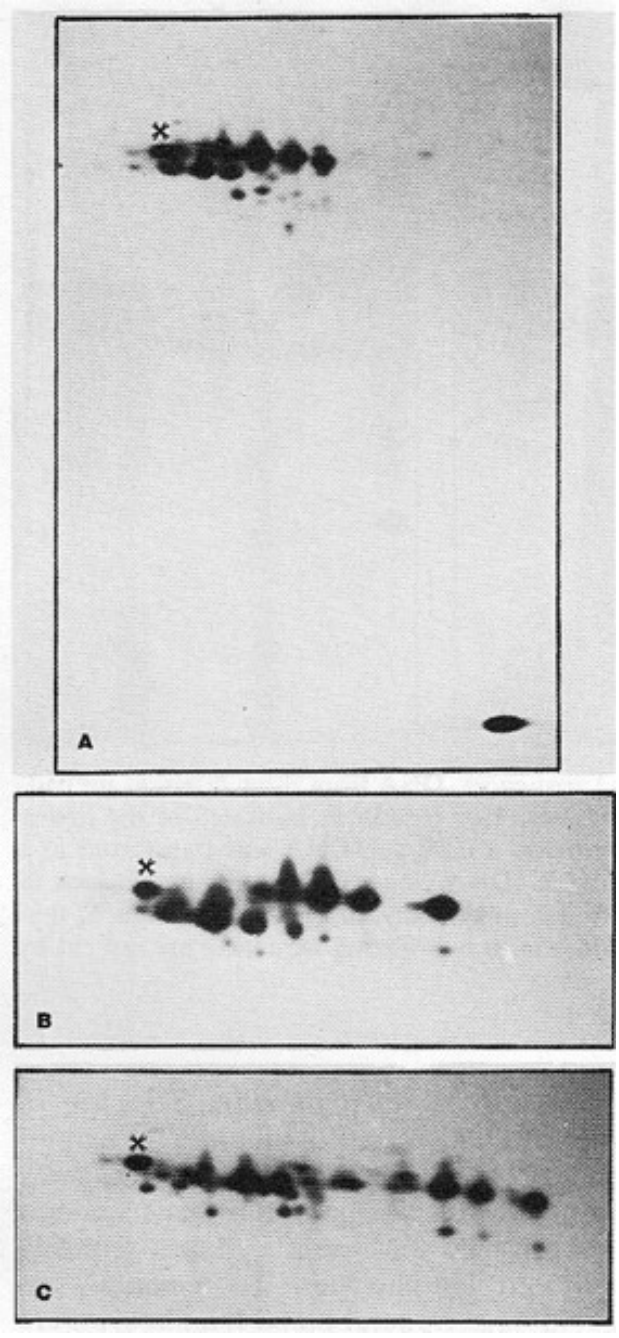

Fig. 2A–C. Analysis of human class I antigens by two-dimensional gel electrophoresis. Class I antigens were isolated by immunoprecipitation with the W6/32 monoclonal anti-HLA antibody and displayed by two-dimensional gel electrophoresis. For detailed methodology see [18]. **A** shows an immunoprecipitate from an HLA-A23, -B7 homozygous individual, the region of the gel from approximately 60,000 daltons to 10,000 daltons being shown. The intense spot at the bottom is β_2-microglobulin. **B** and **C** show immunoprecipitates obtained from HLA-A26, -B56 and HLA-A2, -Bw62 homozygous individuals, respectively. In **B** and **C** only the *HLA* heavy chain region is shown, at a slightly larger magnification than in **A**. The basic end of the gel is oriented toward the *right,* and the position of actin, a common contaminant of such immunoprecipitates, is indicated by *. For further details, see text and [18]

found in man-mouse somatic cell hybrids carrying only the human chromosome 6 [20]. In Fig. 2, the results of such an analysis are given for several different homozygous typing cells. It is apparent that a large number of spots are obtained for each individual donor. This is caused in part by differential sialylation, resulting in the presence of four spots for each specificity. If we take this phenomenon into account, we arrive at a figure of some six class I genes being expressed per haploid genome [18]. Differences in number may be seen according to haplotype, although this matter deserves further investigation. It suffices to say that in the murine system too the complement of class I sequences is thought to show variation according to haplotype. Combined with the results referred to in the previous section, we may conclude that, notwithstanding the presence of a large number of class I sequences per haploid genome, only a small minority are usually expressed in PBLs. Evidence from the analysis of homozygous typing cells suggests that at least 80% of the amount of class I antigen per cell is contributed by the HLA-A, -B antigens [18].

It was already hinted at that this observation may find its cause in the presence of a large number of pseudogenes. Selective pressures to increase the polymorphism of class I antigens may have produced a tendency to duplicate the genes encoding them as a means of increasing variability at the level of the population, as well as that of the individual. A relatively large number of pseudogenes may be the price the organism has to pay for the ability to duplicate given sets of genes. This process may not be altogether harmful nor constitute a great genetic burden since the genes present in the genome, pseudo- or otherwise, are still a substrate for unequal crossing-over events, gene conversions, and the like. Thus the information contained in them can be acted upon by natural selection.

E. Structure of Class I Genes

Using the available cDNA clones as probes, genomic clones have been isolated, and their structure has been determined

([13, 14] and Ploegh et al., in preparation). The structure of a typical class I gene shows an intron-exon organization, which reflects the division of the molecule into what are thought to correspond to functional domains [4]. The signal sequence and the heavy chain domains H1, H2, and H3 are all encoded by separate exons. The membrane-binding region likewise is encoded by a separate exon. A more unusual arrangement is seen in the cytoplasmic part of the molecule, which, in the case of human class I antigens, is interrupted by two introns. It is not clear whether these interruptions imply a functional subdivision at the level of the protein or whether they are fortuitous. It should be kept in mind that for murine class I antigens, highly homologous to their human counterparts, an additional intron is found for the cytoplasmic domain, arguing against a functional subdivision of this part of the molecule as referred to above.

Thus far, little is known about the transcription of *HLA* genes or the factors that exert a regulatory influence. It has been found that interferons increase the expression of HLA antigens by increasing the amount of available mRNA, presumably through action at the transcriptional level [26]. It is of importance to understand these phenomena, since the expression of so-called alien histocompatibility antigens on certain tumors has been postulated being due to the activation of otherwise silent histocompatibility genes. Here, too, a relationship might exist between the ability of the tumor to escape the immune system and the suggestion that "inappropriate" antigens are expressed [27].

F. Variant Antigens

To return to the question of recognition of MHC products by T-lymphocytes, we shall now examine which features on class I antigens may be of importance and can be recognized. As mentioned previously, HLA-A, -B specificities are defined by alloantisera. It was noted by several groups that between HLA-identical individuals cytotoxic T-lymphocytes can be generated that have a specificity for a particular class I antigen, as established by panel and family studies [21, 22]. The conclusion to be drawn from such data is that either variants or subtypes of recognized specificities must exist, or that structures tightly linked to the HLA-A, -B specificities in question can function as targets for cytotoxic T-lymphocytes. A structural analysis of such variant or subtypic antigens has been carried out, and it was found that they were biochemically distinct as well [22, 23]. These initial observations were restricted to two cell lines, one of black and one of oriental racial origin [24]. We carried out similar analyses on a larger panel of white individuals who had been typed for *HLA-A2* by cytotoxic T-lymphocytes and were variant. Our biochemical analysis revealed that these antigens were also biochemically distinct [25]. Peptide mapping and amino-acid sequencing should establish the exact nature of these differences.

These variants or subtypes illustrate the discriminating power of T-lymphocytes recognizing histocompatibility antigens, a feature already well known from the mouse mutant *H-2* work [4, 7]. For subtype identification, the approach outlined in the previous section was used, i.e., isolation of class I antigens by means of a monomorphic monoclonal antibody, followed by separation on a one-dimensional isoelectric focusing gel. This technology affords sufficient resolution to resolve most commonly occurring HLA-A, -B specificities. Although defined by alloantibodies, we can define subtypes without such complex reagents. Such subtypes may not be without clinical significance: If they can be discerned by cytotoxic T-lymphocytes in a cell-mediated lymphocytosis (CML) reaction, it is equally likely that they would be recognized in a clinical transplantation situation. These findings may explain at least in part why matching for HLA-A, -B, -C antigens is of relatively little importance in predicting the outcome of a kidney transplantation. At the same time, techniques such as the present one may identify targets for cytotoxic T-lymphocytes other than those encoded by the *HLA* region. How do these results relate to the problem of human leukemia? Elsewhere in this volume, the reader will find a description of cells transformed with human leukemia virus (HTLV). Such cells express HLA-A, -B, -C

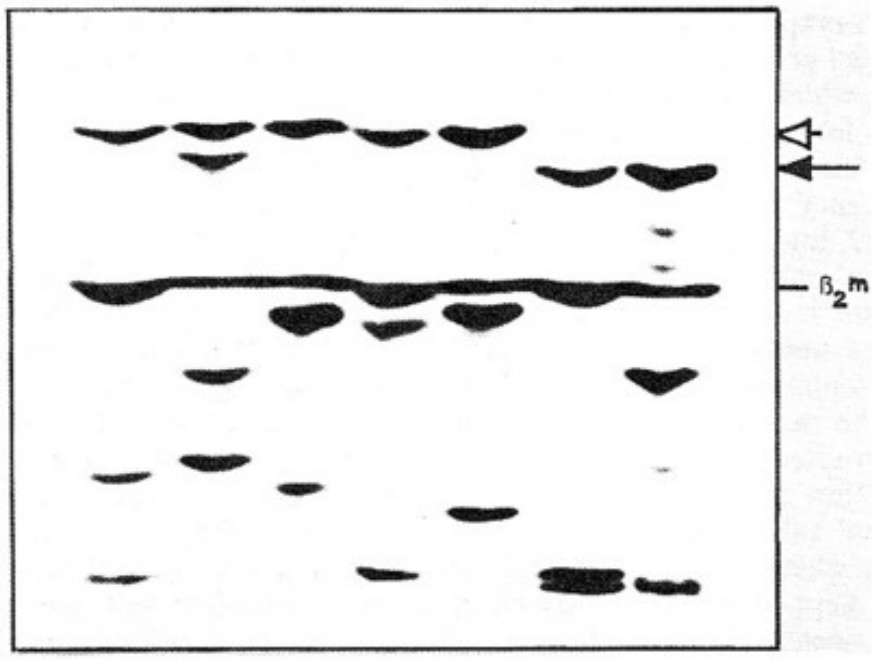

Fig. 3. Analysis of HLA-A2 subtypes by isoelectric focusing. HLA-A, -B, -C antigens were isolated from HLA-A2 positive individuals who could be subdivided according to their reactivity with HLA-A2-specific cytotoxic T-lymphocytes, although indistinguishable serologically. The position of normal A2, as found in 90% of the white human population is indicated by an *arrow*; the position of the minor A2 subtype is indicated by an *open arrow*. The other bands represent other HLA-A, -B specificities present in the different donors. The complexity of such patterns has been reduced by carrying out a neuraminidase digestion on the immunoprecipitates prior to isoelectric focusing. The reader is referred to [19, 22] for further details

antigens other than those found on the original donor lymphocytes. Apparently, there is a link between the transformation event and the expression of HLA antigens at the cell surface. Clearly, a more profound knowledge of how these events interrelate should contribute to our understanding of human leukemia. It would be interesting to analyze biochemically the antigens expressed after transformation by HTLV. Are the HLA-A, -B specificities indeed distinct from those found on the untransformed donor cell, or do they represent unusual post-synthetic modifications? Our description of normal antigens and their subtypes should allow an unequivocal answer to this question.

G. Concluding Remarks

The products of the MHC are amongst the best studied of membrane glycoproteins. We may safely assume that techniques applied to their study will be equally useful for the analysis of other membrane proteins – lymphocyte differentiation antigens and tumor markers. It is equally important to underline once more the central role played by the MHC in the immune response. If the immune surveillance concept is as important as has been assumed so far, and if it is to be understood in detail, the MHC will certainly need further research. There is no shortage of willing hands.

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