

TARGETS FOR KILLER T CELLS

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In tumour immunity as in allograft immunity killer T cells are thought to play an essential role in the response¹. Although more is known of the target antigens in the latter case, the gene products involved have never been precisely defined. The main tool for the investigation of these antigens is the cell mediated lympholysis (CML) assay, in which allogeneic, PHA-induced, blast cells (T cells) were used as targets². Generalizations deduced from this assay can thus be applied to situations involving killer T cells and other foreign tissues, only after due consideration of the phenotypic differences existing between these and PHA-blasts.

It is generally agreed that when a CML test is performed between two randomly selected individuals most of the structures recognized are associated with gene products in the HLA region²⁻⁷. Furthermore it is strongly argued that the HLA-A, -B and -C antigens defined by tissue typing sera and complement are the antigens involved^{3,7-10}. These are hereafter referred to as HLA-SD antigens. Despite these claims the data remains ambiguous in the sense that some foreign HLA-SD antigens elicit weak or zero killer cell response⁷⁻¹⁷.

In addition to these apparent anomalies we and others^{14,18,19} have found that the association of the target antigen with HLA-SD specificities is often incomplete in that not all target cells which carry the appropriate antigen in an unrelated panel are killed and when segregation studies are performed in families the pattern obtained is untypical of HLA-SD antigens which normally segregate in a codominant way. A recessive pattern may, in theory, have accounted for this, because in the mouse there existed a recessive H-2 associated antigen, the haemopoietic resistance (Hh) antigen which segregated in a recessive manner and which was identifiable by CML^{20,21}. However, no such pattern has yet been observed with HLA associated CML antigens in man, neither did it fit our data.

Recent new ideas, which had their conceptual origins in certain mouse experiments have led us to reexamine the assumption that HLA-SD antigens are T cell targets. In these mouse experiments foreign minor histocompatibility antigens were shown to be capable of eliciting killing reactions only if they were accompanied on the target cell by H-2D and K (HLA-SD equivalent) antigens of

the 'self' type. Such antigens were expressed on the effector and target cell and could thus be excluded as the targets in themselves. Nevertheless both 'self' H-2D and/or K and the minor 'foreign' antigen was required for killing and neither alone elicited a killing response^{22,23}.

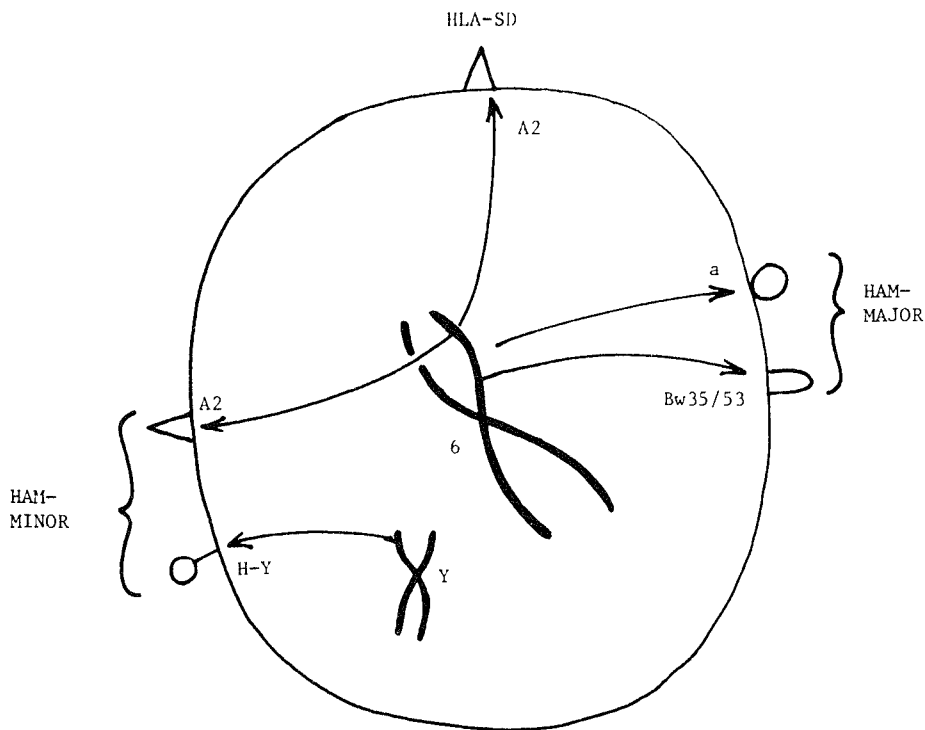
Following these observations an opportunity arose in man to study a similar phenomenon and the results formed the basis of the classification of antigens proposed here.

Initially a patient (Mrs. Reef.), who had been suffering from aplastic anaemia, was studied. She had received multiple blood transfusions and a bone marrow allograft from her HLA identical brother which was ultimately rejected. Thereafter her peripheral blood lymphocytes were shown to be capable of killing target cells from her HLA identical brother thus suggesting that minor histocompatibility antigens were recognized. It was subsequently shown that when these effector cells were tested on a panel of targets from unrelated individuals only male HLA-A2 positive, targets were killed despite the fact that the HLA-A2 antigen was a 'self' antigen present on the cells of Mrs. Reef. herself. This led to the conclusion that the target was a minor histocompatibility antigen (H-Y) encoded on the Y sex chromosome and that killing required the simultaneous expression of the 'self' compatible HLA-A2 antigen on effector and target. Hitherto three examples of this phenomenon have been described and in all cases an HLA-A2 restricted H-Y killing occurred, but in one case this was supplemented by restriction to another 'self' compatible antigen, the HLA-B7 antigen²⁴⁻²⁷.

The methods used for the cell mediated lympholysis (CML) technique have been described in detail elsewhere²⁷. Briefly peripheral blood lymphocytes were sensitized in vitro in one-way MLC's where responder cells, A, were sensitized to irradiated cells B. After 6 days these cells were tested in a 4 hour killing assay on PHA blast cells obtained at 3 days of culture. These had been previously labelled with ⁵¹sodium chromate so that the degree of killing could be measured by the amount of isotope released into the supernatant. Controls included PHA blasts of the original sensitizing cell, B, as positive control and autologous, A, PHA blasts as negative control. Since one of the two major sources of variation in these studies was the ability of PHA blasts to take up and release ⁵¹Cr the results were always corrected for the difference between spontaneous release and maximal release for each individual target cell used. The second major source of variation was the size of the effector cell clone which in turn determined the maximum kill by any effector cell population. In the data shown positive reactions were those which gave percentage kill values

comparable to the positive control value (see for further discussion refs. 18,28).

In two series of experiments an association between antigens recognized in CML and 'self' HLA-SD was demonstrated. In both cases the latter were excluded as the antigens in themselves because the same antigens were present on the effector cells.



SD	Serologically Defined
HAM	HLA-SD Associated at Membrane
MAJOR	HLA Linked
MINOR	Non-linked to HLA

Fig. 1. Schematic representation of a target cell showing two types of targets for T killer cells. The foreign component of the complex target may be encoded outside the major histocompatibility complex (HAM-minor) or in linkage with it (HAM-major). These two examples are further described in the text.

Panel
No.

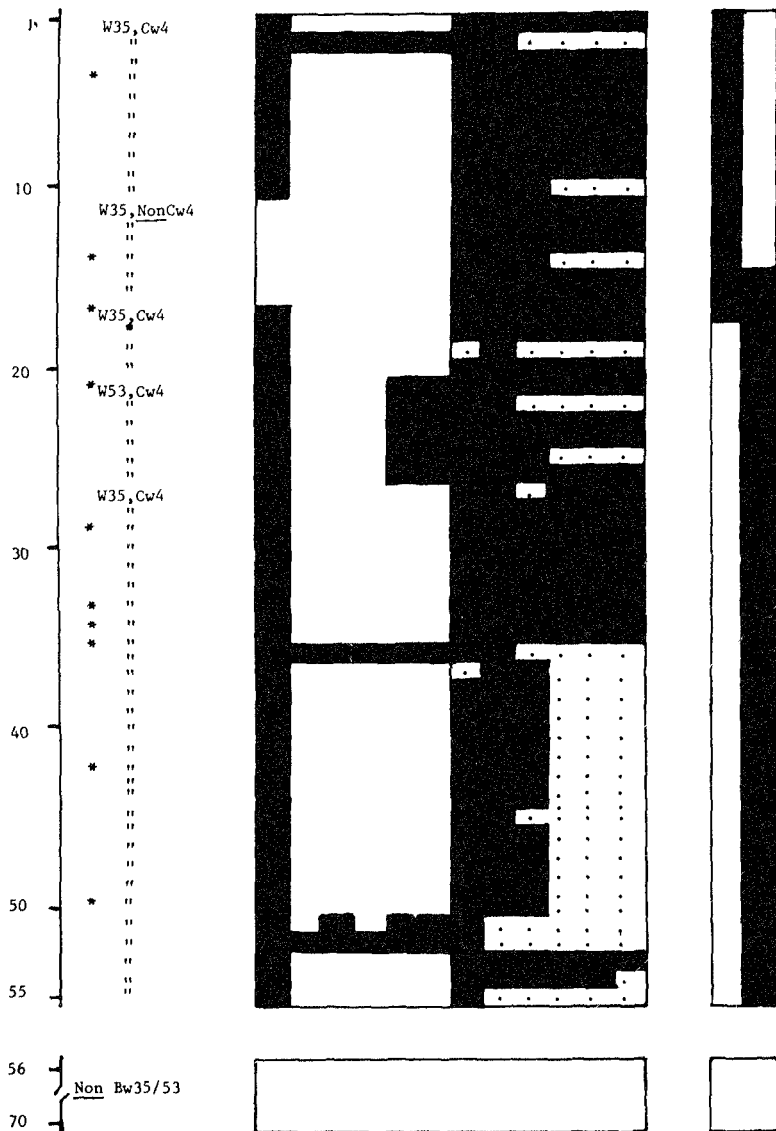
HLA-B₂,C
type




Sera

CML

a⁺ b c d e f g h i j k l

m n



 Positive
 Negative
 Not Tested

* HLA-B locus homozygote or potential homozygote

a ⁺	anti Cw4
b,c	" w51
d	" w51,w52
e,f	" w51,w52,w53
g,h,i	" w51,w52,w53,w35
j,k	" w53,w35
l	" w35
m	anti a
n	" b

Fig. 2. Summary of results obtained from a panel of 70 individuals who had been typed with various antisera which recognized the Bw35 group of antigens, and with two CML effector cell preparations (m and n) which recognized Bw35 associated antigens, a and b.

In an attempt to clarify what at first appeared to be confusing observations, a hypothetical classification of antigens is now proposed. In general it could be said that two types of antigen exist both being associated with 'self' HLA-SD antigens at the cell membrane level. Under no circumstances is the association referred to meant to imply molecular interaction between the HLA-SD determinant and the other determinant concerned, this question remains open. The term is used solely to imply that a simultaneous (associated) recognition of both antigens must occur before killing takes place. Antigens which are HLA Associated at the Membrane are abbreviated to HAM antigens. These can be subdivided into antigens which are encoded outside the HLA region, as in the case of minor histocompatibility antigens from which we derive the term HAM minor antigens, or, alternatively they can be in linkage with the HLA complex and are thus products of the major histocompatibility complex and because of this we derive the term HAM major antigens (see Fig.1).

An example of a HAM minor antigen was one which was encoded on the Y chromosome. It was recognized in association with the 'self' compatible HLA-A2 antigen and it occurred in three independent cases, one of which also involved HLA-B7. These results were described elsewhere²⁴⁻²⁶.

A possible example of a HAM major antigen was an antigen which was recognized by effector cells raised in vitro between HLA-SD identical, but genotypically HLA non-identical, family members. These data have been mostly described elsewhere²⁷. Family segregation studies were compatible with the view that the antigens detected by CML (a and b) were encoded in the HLA region. When tested upon an extensive panel of individuals who typed positively or negatively for HLA-Bw35 and/or its W4 associated antigen, Bw53, only individuals who carried these HLA-SD antigens were killed. Fifty-five individuals who carried Bw35 and/or Bw53 were tested and it was remarkable that cells from all 55 individuals were killed by either anti-a or anti-b. None of the 17 individuals who were negative for these HLA-SD antigens were positive and although this rendered the possibility of non Bw35/53's being killed highly unlikely, further confirmation of this awaited the testing of a larger panel (Fig. 2). The reaction pattern obtained with the effector cells, anti-a and anti-b, failed to correlate with any of the patterns obtained with the available typing sera. These CML defined antigens therefore appeared to fulfill the criteria for HAM major antigens in that they were encoded in the major histocompatibility complex and were seen only in association with specific 'self' compatible HLA-SD antigens.

One interpretation of this data is that HAM major antigens follow the same rules of associative recognition as have already been demonstrated for HAM minor antigens and that a similar complex antigen is formed by the products of two separate cistrons which are in this case both encoded in the HLA region. In suggesting this it should be stated that we cannot completely exclude the possibility that these CML specificities are in fact represented within the HLA-SD typing sera used, most of which are clearly multivalent (Fig. 2). An alternative interpretation is that T killer cells recognize a carrier molecule plus an appropriate hapten (Bw35/53) whereas the antisera only recognize the hapten portion of the molecule.

The view that HLA-SD antigens in themselves acted as target antigens in CML was lent further support by some recent experiments in man²⁹. In general, however the data remain inconclusive, it was first suggested that such antigens were associated with killing when it was shown in the mouse that target cells which had been previously incubated with anti-H-2 sera subsequently became resistant to killing³⁰. Furthermore mouse cell lines which failed to express the molecules carrying H-2D and K antigens were neither capable of sensitizing nor capable of being killed in CML assays, thus implying a specific role for these molecular structures in T cell killing, despite the possibility that they may not be targets in themselves^{31,32}.

In man discrepancies have been recorded regarding the behaviour of HLA-SD antigens as targets in so far that not all antigens performed equally well in the generation of effector cells. It was suggested that the antigens HLA-A2, -B7, -B8, -B12, -Bw35 were strong immunogens whereas others were weak immunogens e.g. HLA-A1, -A3, -B11, and -Aw19¹⁸. Such observations were originally interpreted to support a suggestion that target antigens constituted an integral part of the 'backbone' structure of the HLA-SD molecules which were seen exclusively by killer T cells^{16,18}.

HLA-D region products have hitherto been considered by most workers to be incapable of behaving as targets for T killer cells^{8,9,12,33,34}. However most attempts to demonstrate HLA-D target antigens have used 'conventional' CML assays of the type described here. Three pieces of data suggested that this may have been an inappropriate technique. Firstly, in the mouse it was possible to demonstrate CML to all regions of the H-2 complex, including the H-2 IA region (HLA-D equivalent), providing the appropriate technical modifications were introduced³⁵⁻³⁷. Secondly, gene products of the Mls locus in the mouse, which induced strong proliferative responses in allogeneic cell mixtures consistently failed to behave as targets in conventional CML assays but were thought to be detectable by the so called 'cytostasis' assay in which macrophage monolayers were used as targets³⁸. Thirdly, it was recently demonstrated in man that in CML assays in which blood monocyte-macrophage cells were used as target cells, HLA-DR associated gene products could behave as antigens³⁹.

Finally, with regard to the relevance of this discussion to tumour immunity one should mention phenomena in mouse tumour models where, in some cases, changes in the H-2D and K structures have been observed^{40,41}. In others H-2D and K restricted CML reactions to tumour associated antigens have been demonstrable^{42,43}. However, exceptions to this latter phenomenon have also been described⁴⁴. We feel therefore that it is legitimate to suggest that tumour associated antigens in man may in some cases form antigen complexes in association with 'self' HLA-SD antigens which are recognized by the host's killer T cells.

SUMMARY

"Conventional" CML assays in man have been widely used to investigate antigens recognized by killer T cells. Although the detection of these antigens appears to be dependent on expression of the molecular structures which carry HLA-SD (HLA-A, -B and -C encoded serologically defined) antigens there is no clear evidence that these behave as targets in themselves. Two hypothetical categories of targets detected by killer T cells are proposed both of which

are HLA-SD Associated at the cell Membrane level (HAM). The allogeneic or foreign portion of the HAM antigen may be encoded outside the major histocompatibility complex and such a complex is termed a HAM minor antigen, and an example is given of the HLA-A2 associated H-Y antigen. Alternatively it may be encoded in linkage with the major histocompatibility complex and is thus termed a HAM major antigen. A possible example of this is an HLA encoded antigen which is associated with the HLA-Bw35 group of antigens. If such a classification is generally applicable, it may explain some of the apparent ambiguities regarding HLA-SD antigens and targets for killer T cells.

ACKNOWLEDGEMENTS

This work was in part supported by the Dutch Foundation for Medical Research (FUNGO) which is subsidized by the Dutch Organization for the Advancement of Pure Research (ZWO), the Dutch Organization for Health Research (TNO) and the J.A. Cohen Institute for Radiopathology and Radiation Protection (IRS).

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