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THE IMMUNOGENETICS OF CHRONIC GRAFT VERSUS HOST DISEASE AND ITS RELEVANCE FOR THE GRAFT VERSUS LEUKEMIA EFFECT

Jon J. van Rood, Els Goulmy and Aad van Leeuwen

Department of Immunohaematology and Blood Bank, University Hospital, Leiden, the Netherlands

INTRODUCTION

The debate whether (chronic) graft versus host disease (cGvHD) after bone marrow transplantation reduces the chance of leukemic relapse is certainly not definitively settled, but the majority of those involved in bone marrow transplantation including our own group feel that this is the case (Weiden et al. 1981; Zwaan et al. 1986; Gale et al. this conference). Assuming this indeed to be true, an insight in the mechanism by which cGvHD diminishes leukemic relapse could be of importance not only for the management of leukemia but also of other malignancies.

Good experimental evidence exists that the occurrence of cGvHD if donor and recipient are MHC identical, is due to differences for minor H(istocompatibility) or non-MHC antigens (Goulmy et al. 1985), although viral and other infections almost certainly play an additional role as well (Gratama et al. 1987). During the last years we have performed a systematic study to answer the question whether donor bone marrow derived anti-recipient cytotoxic T lymphocytes (CTLs) might occur during cGvHD. This turned out indeed to be the case (Goulmy et al. 1986). Using such CTLs it was possible to do population and (limited) family studies. The data obtained so far are compatible with the assumption that the determinants which are recognized are coded for by genes which are located on chromosome 6 teleomeric from HLA-A. We will review the available data and speculate on a mechanism by which cGvHD could control leukemic relapse.

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MATERIALS AND METHODS

Peripheral blood lymphocytes

Peripheral blood lymphocytes were obtained from AML, ALL, CML and AA patients in the departments of Haematology and Pediatrics of the University Hospital in Leiden before and after transplantation.

Cell mediated lympholysis

Cell mediated lympholysis (CML) tests were performed as described previously (Goulmy 1982). In brief, for the primary in vitro sensitization, equal amounts of responder cells and 2000 Rad gamma irradiated stimulator cells were incubated for 6 days at 37°C, 5% CO_2 in a well humidified incubator. Thereafter, cytotoxic T cell lines were grown (by the use of specific irradiated feeder cells and IL-2), tested for specific cytotoxic activity and subsequently stored in small aliquots in liquid nitrogen.
For the performance of the CML assay, CTLs or cytotoxic T cell lines were incubated with ^{51}Cr labelled target cells (phytohemagglutinin stimulated blast cells) at six effector cell : target cell ratios for 4 hrs. at 37°C. Cytotoxicity (i.e. the amount of isotope released from ^{51}Cr labelled target cells) was determined and calculated according to the described method (Goulmy 1982).

Protocol

As responder cells post-transplant patient's PBLs (i.e. the donor bone marrow derived PBLs) were used. As stimulator cells, the liquid nitrogen stored PBLs obtained from the patient before transplantation were used. Because all patients studied were HLA-A,B,C,DR identical and MLC negative with their bone marrow donor, the responder cells could only be "sensitized" (in vivo) against determinants which were coded for by genes outside the HLA-A/DR region on chromosome or any of the other chromosomes

RESULTS

Table 1 summarizes the results obtained in the first family tested (HA). Donor and patient, a 24 year old male

transplanted in complete remission from AML and now suffering from cGvHD were HLA-genotypically identical (ad) Likewise three healthy siblings shared the haplotypes ac Nevertheless, the cells of the donor (obtained from the donor before transplantation) and the sibling 03 failed to react with the CTLs raised from the patient's PBLs obtained after bone marrow transplantation In other words the CTLs recognized a determinant which was coded for by a gene outside the HLA-A/DR region

FAMILY HA

	Haplotypes	% lysis
Father	ab	+91
Mother	cd	+84
Patient (pre-transplant)	ad	+82
Donor	ad	-3
Sibling 03	ac	+6
" 04	ac	+85
" 05	ac	+92

Haplotypes a A2,Bw62,Cw3,DR4
b Aw24,Bw35,Cw4,DR7
c A3,Bw35,Cw4,DR1
d A2,B27,Cw1,DR1

TABLE 1 CTL reactivity against a non-HLA-A,B,C DR antigen in a patient HA suffering from cGVHD and his familymembers

Similar CTLs could be found in 7 out of 9 patients suffering from cGvHD (including patient HA) in 1 out of 4 patients suffering from acute GvHD and in none of the 8 patients without GvHD

The HLA-A2 restricted CTLs which were obtained from four different patients were tested against a panel of lymphocytes obtained from 120 unrelated healthy HLA-A2 positive donors Table 2 depicts the positive and negative results obtained HA1 is included in HA2, that is HA2 is always present if HA1 is positive HA4 and HA5 are identical in this panel There is a significant association between HA1 and HA2 as well as HA4 and HA5 The association between HA2 and HA4 and HA5 is not significant

The segregation pattern in five family studies was compatible with the assumption that the gene or genes coding for HA1,2,4 and 5 were in close linkage with HLA, although crossovers appear to occur as well (data not shown) On the basis of an informative crossover between HLA-A and -B, the HA gene(s) appear(s) to be located teleomeric from HLA-A

MINOR H ANTIGEN TYPING AT THE POPULATION LEVEL

Tested		HA-1	HA-2	HA-4	HA-5
N	64	pos	pos	pos	pos
N	23	pos	pos	neg	neg
N	13	neg	pos	neg	neg
N	6	neg	neg	pos	pos
N	6	neg	neg	neg	neg
N	112	all HLA-A2 positive			

TABLE 2 Reactivity of CTLs anti-HA1, anti-HA2, anti-HA4, anti-HA5 against a panel of 120 HLA-A2 positive individuals

Table 3 summarizes the typing data for HA1,2,4 and 5 in 64 donor-recipient pairs It is clear that a significant majority of the mismatches for HA occurred in the patients who suffered from cGvHD Furthermore these data support the notion that the great majority of HLA identical siblings are also HA identical, which would be in accord with the notion that the HA system is coded for by genes located on chromosome 6 near HLA

MINOR H ANTIGEN TYPING OF HLA-IDENTICAL SIBLINGS

Donor/recipient pairs	Typing for HA-1, -2, -4 and -5	
	Identical	non-identical
recipients without GvHD	20	0
recipients with acute GvHD	26	3
recipients with chronic GvHD	4	8

X_2^2 24,76

$p = < 10^{-6}$

TABLE 3 HA1,2 4 and 5 typing data in 64 donor-recipient pairs of a bone marrow transplantation in relation to the occurrence of cGvHD

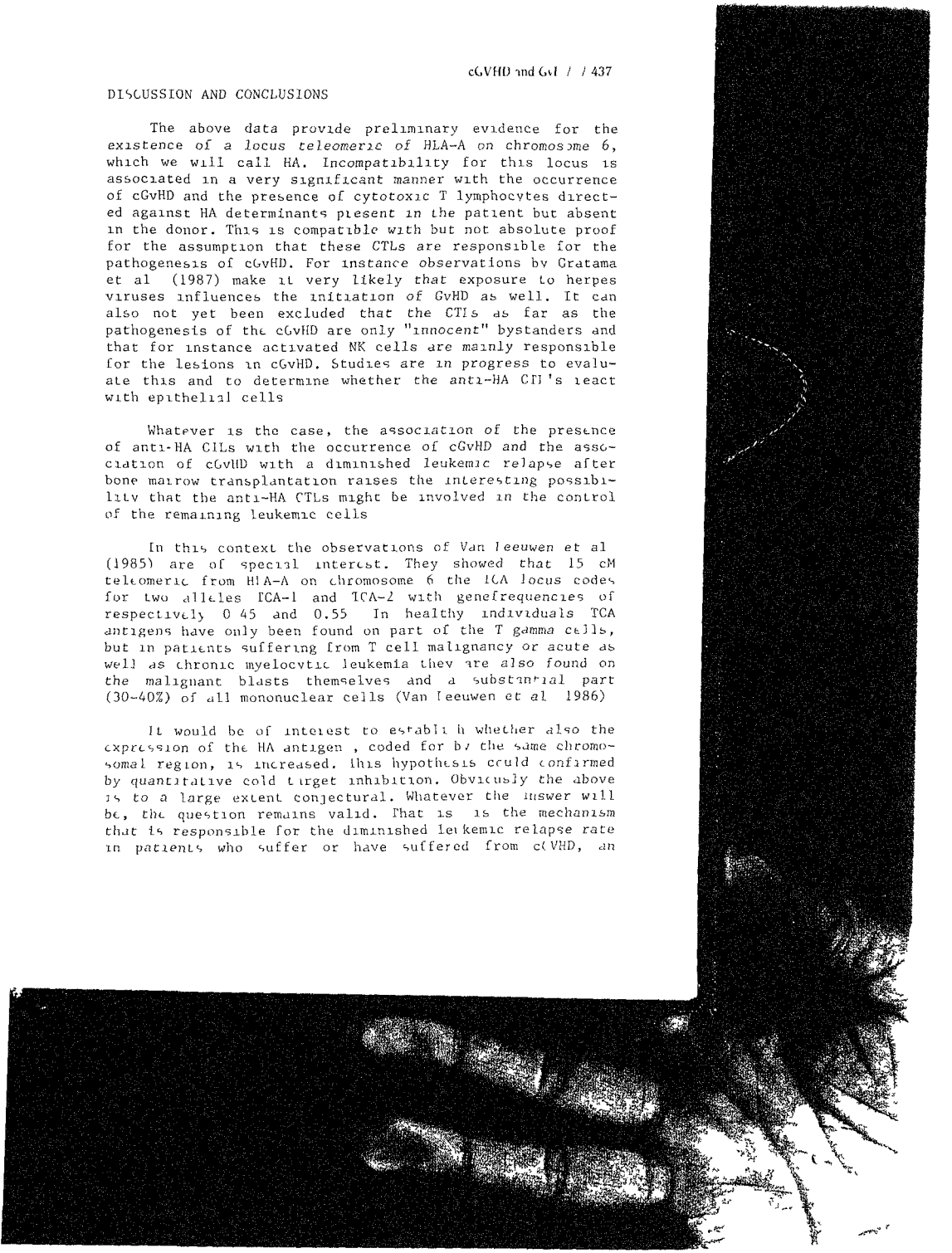
DISCUSSION AND CONCLUSIONS

The above data provide preliminary evidence for the existence of a *locus teleomeric of* HLA-A on chromosome 6, which we will call HA. Incompatibility for this locus is associated in a very significant manner with the occurrence of cGvHD and the presence of cytotoxic T lymphocytes directed against HA determinants present in the patient but absent in the donor. This is compatible with but not absolute proof for the assumption that these CTLs are responsible for the pathogenesis of cGvHD. For instance observations by Gratama et al (1987) make it very likely that exposure to herpes viruses influences the initiation of GvHD as well. It can also not yet been excluded that the CTLs as far as the pathogenesis of the cGvHD are only "*innocent*" bystanders and that for instance activated NK cells are mainly responsible for the lesions in cGvHD. Studies are in progress to evaluate this and to determine whether the anti-HA CTL's react with epithelial cells

Whatever is the case, the association of the presence of anti-HA CTLs with the occurrence of cGvHD and the association of cGvHD with a diminished leukemic relapse after bone marrow transplantation raises the interesting possibility that the anti-HA CTLs might be involved in the control of the remaining leukemic cells

In this context the observations of Van Leeuwen et al (1985) are of special interest. They showed that 15 cM teleomeric from HLA-A on chromosome 6 the TCA locus codes for two alleles TCA-1 and TCA-2 with genefrequencies of respectively 0.45 and 0.55 In healthy individuals TCA antigens have only been found on part of the T gamma cells, but in patients suffering from T cell malignancy or acute as well as chronic myelocytic leukemia they are also found on the malignant blasts themselves and a substantial part (30-40%) of all mononuclear cells (Van Leeuwen et al 1986)

It would be of interest to establish whether also the expression of the HA antigen , coded for by the same chromosomal region, is increased. This hypothesis could confirmed by quantitative cold target inhibition. Obviously the above is to a large extent conjectural. Whatever the answer will be, the question remains valid. That is is the mechanism that is responsible for the diminished leukemic relapse rate in patients who suffer or have suffered from cGVHD, an



immunological one and if so, what are the effector cells and the target determinants. In this context it would be of interest to determine whether PBL's from patients who have recovered from cGVHD have demonstrable anti leukemic blast cell activity.

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INTRODUCTION

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