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MHC RESTRICTED CYTOTOXIC T LYMPHOCYTES RECOGNIZE A HUMAN MINOR TRANSPLANTATION ANTIGEN.

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Summary

Recently we demonstrated, that post-transplant lymphocytes from a bon-marrow transplanted A.M.L. patient, suffering from severe GvHD, exhibited strong cytotoxicity in CML against his own pre-transplant lymphocytes. Since the patient has been transplanted with bone-marrow from his HLA identical female sibling, it could be concluded that the transplanted lymphocytes differed in a non HLA-A to -DR antigen from the patient. The initiative experiments comprising a panel-analysis of unrelated individuals, indicated that patients post-transplant cytotoxic effector cells were recognising an HLA associated minor transplantation antigen (designated "HA").
We report here an extension of our first observation. The patients post-transplant cytotoxic effector cells were tested against a comprehensive number of a) randomly chosen unrelated healthy paneldonors; b) relatives of the positively lysed paneldonors and c) several bone-marrow donor/recipient combinations. Our results suggest that the cytotoxic effector cells, which are derived from a patient suffering from severe GvHD, recognize one (or more) minor transplantation antigen(s) in association with the three self class I HLA antigens of the patient. The non HLA minor histocompatibility antigen(s), showing different gene frequencies for the three restricting elements, segregated in many but not all families in a codominant way.

Introduction

Graft versus host disease (GvHD) occurs in recipients grafted with bone marrow from HLA identical sibling. Thus, histocompatiblity antigens other than HLA are apparently playing a role in the occurence of moderate, severe and lethal GvH. To our knowledge, only few reports sofar suggest the impact of polymorphic genetic systems other then HLA on the occurence of GvHD in man (1,2). Our earlier report described the presence of cytotoxic effector cells (CTLs) in a patient suffering from severe GvHD(3). Since the latter CTLs appeared after grafting it suggests in itself that the HLA identical donor and recipient differed for a non HLA transplantaion antigen. We extended the search to identify this non HLA antigen. In this report, we demonstrate that the patients' CTLs are recognizing one (or more) target determinants in a MHC restricted fashion. The population frequency of the minor transplantation antigen and the segregation patterns will be discussed.

Materials and Methods

The Cell Mediated Lympholysis (CML) assay used has been described in detail (4). The effector cells used throughout the whole study were patients' post-transplant peripheral blood lymphocytes sensitized in vitro for 6 d. with pretransplant lymphocytes (designated; CTLs α HA). These cytotoxic effector cells were tested against or ^{51}Cr labeled target cells in a 4 h. assay; the amount of isotope released from the

target cells was determined and calculated according to the method described earlier (4). All experiments were repeated at least three times and carried out in 6 effector: target ratios.

Results and discussion

Table 1 shows the pattern of lysis in CML in patients' own family"HA". As already mentioned, patients' CTLs were able to lyse his own pre-transplant lymphocytes but not the lymphocytes of his bone-marrow donor; additionally absence of lysis was repeatedly shown against the lymphocytes of one (i.e. child 3) out of three HLA identical siblings. In an attempt to identify the target determinant, we tested CTLs α HA against a large number of randomly chosen unrelated individuals. The results in table 2 illustrate that the new non HLA target determinant could only be recognized in the presence of the three self class I antigens of the patient. The results of the panel study allows us to say that the HLA-A2 restricted determinant demonstrates a high gene frequency, in contrast to the two other restricting elements (i.e. B27 and Bw62) which apparently show much lower frequencies of the new HLA determinant. In an attempt to determine whether or not the cytotoxic determinant was genetically defined, families of panelmembers which were lysed and in addition relatives of several bone-marrow transplanted patients were investigated with the use of CTLs α HA. In all families from healthy panelmembers the HLA restricted minor transplantation antigen showed Mendelian segregation (data not shown). Only two families were aberrant. Those were confined to the patients' family (see table 1) and to a family of another bone-marrow transplanted patient. Table 3 shows that the father (who in fact donated the bone-marrow to his HLA compatible child) does not carry the minor transplantation antigen "HA" in contrast to the bone-marrow recipient who was apparently positive for "HA" prior to transplantation. The grafted patient is suffering from severe GvHD.

Although our knowledge of minor histocompatibility antigens, which might play a role in GvHD in man, is very limited, our results sofar indicate that it might be of interest and is possible to identify such minor transplantation antigens. The minor transplantation antigens which are coded for by genes distributed all over the genome, influence the success of bone-marrow transplantation. At the moment it is possible, using cytotoxic T-cell lines derived from patients' post-transplant peripheral blood lymphocytes, to type for one of these minor transplantation antigens. Matching for these minor transplantation antigens prior to transplantation might be of clinical importance.

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Table 1 Patterns of lysis in CML in patients' own family "HA"

Target cells	HLA genotypes							% lysis
	A		B		C		DR	
Father	2	w24	w62	w35	w3	w4	4,7	+91
Mother	2	3	27	w35	w1	w4	1,1	+84
Patient(pre-trpl.)	2	2	27	w62	w1	w3	1,4	+82
Donor	2	2	27	w62	w1	w3	1,4	- 3
Child 3	2	3	35	w62	w3	w4	1,4	+ 6
Child 4	2	3	35	w62	w3	w4	1,4	+85
Child 5	2	3	35	w62	w3	w4	1,4	+92

Table 2 Evidence for one(or more) HLA - restricted cytotoxic determinant (s)

	Target cells			
	HLA-A2	HLA-B27	HLA-Bw62	Others
CML +	91*	2	18	0
CML -	4	13	8	55

* Number of target cells of paneldonors tested.

Table 3 Pattern of lysis of the HLA restricted minor transplantation antigen in family "He"

Target cells	HLA genotypes				% lysis
	A	B	C	DR	
Father (bone-marrow donor)	1,2	7,8	w7	2,3	+ 9
Mother	1,2	7,8	w7	2,3	+69
Patient(pre-trpl.)	1,2	7,8	w7	2,3	+79
Child 2	1,1	8,8	w7	3,3	+10
Child 3	2,2	7,7	w7	2,2	+71