Histocompatibility Antigens and T Cell Responses to Leukemia Antigens

Oliver, R. T. D., Lee, S. K.

Institute of Urology, 172 Shaftesbury Avenue, and National Institute for Medical Research, London, Great Britain

Despite the enormous number of publications on the subject, there is little solid evidence in man for tumour specific antigens which are both antigenic and immunogenic compared with the ease to which such antigens have been demonstrated for transplantable tumours in experimental animals. The failure to demonstrate such antigens in a recent study of spontaneously occurring tumours in laboratory mice has been interpreted as evidence against the existence of tumour antigens in spontaneous tumours in man. Such data cannot exclude the possibility that there may be weakly immunogenic antigens undetected by the systems under test.

The use of immunological stimulation with one molecule to augment response to an otherwise non-immunogenic molecule is not new. In experimental animals the use of carrier molecules to stimulate antibody response to hapten are standard procedures and the interdependence of T and B cells in these responses is well documented. It is usual in these systems for the hapten to have to be attached to the carrier molecule. In contrast for cellular responses to transplantation antigen which also have been shown by Eijsvoogel [1] to require two signals (i.e. a serologically defined HLA antigen and a lymphocyte activating HLA D antigen) it has been clearly demonstrated that it is not necessary for the two signals to be presented on the same stimulating cell. Using three cell experiments i.e. two stimulators, one differing for HLA A, B and C antigens and one differing for HLA D antigens from the responder, Eijsvoogel [1] demonstrated that the two types of antigen can be present on separate cells and still produce cytotoxic lymphocytes which retain specificity for the target HLA antigen but did not react to the cell which only carried the lymphocyte activating HLA D antigen.

Applying this principal to patients with acute leukemia, Sondell et al. [4] demonstrated that HLA identical siblings of patients with acute leukemia developed significantly greater cytotoxicity against the patient's leukemia blast cell if primed in vitro with the patient's blast and a third party allogeneic lymphocyte, than when they were primed with the patient's blast cell alone. Zarling et al. [5] reported one patient where it was possible to generate significant cytotoxicity of remission lymphocytes against autologous blasts by priming with the autologous blast cell mixed with allogeneic lymphocytes. Lee and Oliver [2] recently applied this technique to study patients receiving either BCG or BCG plus allogeneic blast cells as remission maintenance immunotherapy. They found that 10 out of 14 patients studied were able to

generate significant T cell mediated cytotoxicity against the specific autologous blast cell when primed in vitro with the specific blasts in presence of a third party allogeneic cell. This cytotoxicity was specific for the leukemia as no cytotoxicity was demonstrable against PHA transformed autologous remission lymphocytes (or autologous remission bone marrow in the one case tested). Investigation of the nature of the antigen on the third party helper cell using lymphoblastoid cell lines showed that the cytotoxic cells could only be generated if the helper cell was HLA D antigen different from those of responder but not if they were only different for HLA A and B locus antigens. Using this technique it was not possible to use cross killing experiments against allogeneic blast cells to investigate whether the target antigen was present on allogeneic blast cells. This was because the use of a third party allogeneic cell in the priming mixture always led to production of anti-HLA cytotoxic cells in addition to anti-leukemia cytotoxic cells. However results from four indirect approaches (in vitro priming with allogeneic blast cells and testing on the autologous blasts [2], cross over experiments with pairs of patients [2], absence of any difference in the level of cytotoxicity in patients who have received BCG or BCG plus allogeneic blast cell immunotherapy [3], cold target inhibition experiments [2]) suggest that the target antigen is not present on allogeneic blasts.

Recent experiments have been directed to investigate the nature of the target antigen on the autologous blast cell. To exclude the possibility that the target antigen was a normal myeloid or T or B lymphocyte differentiation antigen, anti-leukemia cytotoxic cells were tested against autologous remission bone marrow and autologous remission T or B enriched lymphoid sub populations (see Table 1). There was no evidence from these experiments for the target antigen being a normal differentiation antigen, though to be absolutely certain of this it would be necessary to use purified stem cells from remission bone marrow which would be technically extremely difficult to do.

The experiments in Table 2 attempt to exclude the possibility that the cytotoxicity demonstrable in this assay was due to cross reactivity with normal HLA antigens generated by priming in the presence of an allogeneic

Table 1. Evaluation of normal T and B lymphocytes as targets for anti leukemia CML effector cells

Priming stimulus	Targets					
	Autologous T lymphocytes		Autologous B lymphocytes		Autologous leukemia blasts	
	1	2	1	2	1	2
Autologous B lymphocytes	1[a]	ND	3	ND	0	ND
Autologous B lymphocytes + Allogeneic lymphocytes	0	ND	5	ND	0	ND
Autologous leukemia blasts + Allogeneic lymphocytes	0	0	8	0	21	13

[a] % specific ^{51}CR release

Table 2. Specificity studies on anti-leukemia effector cells

	Targets							
	Autologous remission lymphocyte		Autologous leukemia blast		Allogeneic lymphocyte		Daudi	
	a	b	a	b	a	b	a	b
GP	0	0	0	25	0	0	ND	20
EB	0	0	0	0	0	0	ND	40
JK	0	0	0	46	0	0	ND	70
TW	0	0	0	25	0	0	ND	90
MB	2	0	0	44	13	8	ND	45
LF	ND	0	ND	18	ND	0	ND	23

[a] Remission lymphocytes cultured 6 days without priming.
[b] Remission lymphocytes primed with autologous blasts plus Daudi.

lymphocyte. The experiments shown make use of the fact that Daudi does not express serologically defined HLA A, B or C locus antigens, but can help in recognition of autologous leukemia antigen. These experiments which demonstrate that effector populations with anti leukemia activity do not kill allogeneic normal cells are in contrast to those of Zarling et al. [6]. They reported two patients with hairy cell leukemia where cytotoxic cells produced by priming the patient's lymphocytes with a pool of allogeneic normal cells generated significant cytotoxicity against their own leukemia cell. It is possible than an adequate number of normal cells were not tested using our assay or that the target antigen in AML is different from that in hairy cell leukemia. Further investigation of this is clearly required to clarify the difference.

Conclusions

The use of allogeneic cells to facilitate recognition of a non-immunogeneic leukemia antigen opens up new avenues for development of immunotherapy in human tumours. The fact that the target antigen has not been demonstrated on allogeneic tumours invalidates most of the recent studies of immunotherapy using allogeneic tumour cells.

References

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