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Absence of correlation between graft-versus-host associated immunosuppression and cytotoxic T cell activity in response to major histocompatibility antigens

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Abstract. Studies in mice suggest that the T cell subset involved in graft-versus-host-reaction (GvHR) across the major histocompatibility complex (MHC) depends on the class of MHC antigens recognized by the donor cells. However, the correlation between phenotype and function is not absolute. Using a functional approach, we investigated in a parent →F_1 hybrid model differing at the whole MHC, whether graft-versus-host (GvH) associated immunosuppression was correlated with donor cytotoxic T cell activity. The immunodeficiency was tested by the ability of the F_1 mice to generate a cytotoxic T cell response against trinitrophenyl-modified syngeic cells (TNF-self) or an alloantigen. F_1 specific parental cytotoxic T cells, generated in vitro, induced less immunosuppression than naive parental cells. Specific in vivo priming increased the cytotoxicity of parental spleen cells, but decreased their capacity to induce GvH-associated immunosuppression. In contrast, nonspecific priming resulted in the usual immunodeficiency. In conclusion, there was no correlation between GvH-associated immunosuppression and cytotoxic T cell activity of the parental cells.

Key words: Graft-versus-host – Cytotoxic T cell – Immunodeficiency

On transfer of parental T cells into non irradiated, adult F_1 recipient mice, the recognition of allogenic class I plus class II major histocompatibility complex (MHC) antigens on host cells can result in a graft-vs-host (GvH) reaction characterised by a profound immunodeficiency [6, 11, 15, 17]. It has been reported that in this model, both $CD4^+$ and $CD8^+$ parental T cells are necessary to induce GvH-associated immunosuppression [5, 14]. However, the correlation between phenotype and function or phenotype and MHC class specificity is not absolute and $CD8^+$ lymphocytes define both cytotoxic and suppressor cells. Using a functional rather than a phenotypic approach, we investigated whether GvH associated immunosuppression was correlated with the cytotoxic T-cell activity of parental cells.

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Materials and methods

Mice. Male mice 6 to 8 weeks of age were used in all experiments. C57BL/10 (B10), B 10.BR (BR) mice were used as the donors and (C57 BL 10 × B 10.A) F_1 [(B10 × B10A) F_1] (C57 BL 10 × B10.BR) F_1 [(B10 × BR) F_1] and (B 10.BR × B 10.D2) F_1 [(BR × D2) F_1] were used as the GvH recipients.

Sensitization. Sensitization of parental cells against the F_1 hybrids was performed in vitro and in vivo. In vitro generation of cytotoxic T lymphocytes (CTL) was performed as previously described [10]. Briefly, CTL were generated in 2 ml macrocultures consisting of 4×10^6 B10 spleen cells and 1×10^6 irradiated (B10 × B10 A) F_1 spleen cells. The effectors were tested 5 days later in a 4-h ^{51}Cr release assay on Con A-stimulated spleen cells blasts. In vivo immununization was performed by 3 intraperitoneal (i.p.) injections of BR mice with 10^7 B10 spleen cells. The spleen was removed 1 week following the last injection, and the CTL activity was tested as previously mentioned.

Injection of F_1 mice with parental spleen cells. F_1 mice were injected intravenously (i.v.) with either 4×10^7 normal parental spleen cells or 4×10^7 alloreactive T cells generated in vitro or in vivo.

GvH-associated immunosuppression. Immunosuppression was assessed 2 weeks following the induction of the GvH reaction by the ability of spleen cells to generate CTL against trinitro-phenyl-modified syngeneic cells (TNP-self) or allogeneic cells as previously described [10].

Results

Failure of in vitro generated CTL to induce GvH-associated immunodeficiency

We injected 4×10^7 B10 spleen cells obtained after in vitro culture with (B10 × B10A) F_1 cells i.v. into non-irradiated (B10 × B10A) F_1 mice. Spleen cells from these F_1 hybrid

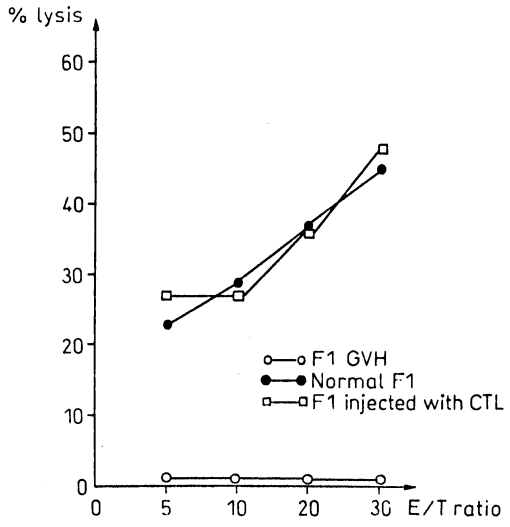

Fig. 1. Inability of in vitro generated CTL to induce GVH-associated CTL suppression. B10 CTL generated against B10A were injected into (B10 × B10A) F_1 and CTL assays were performed 2 weeks later against (B10 × B10A) F_1 TNP-self

mice were tested 2 weeks later for their potential to generate a CTL response in vitro to irradiated TNP-self or allogeneic cells. The data in Fig. 1 show that the CTL reactivity against TNP-self was not reduced. In contrast, no anti-TNP-self CTL was detected in the F_1 recipients that had been injected with 4×10^7 normal parental spleen cells. Similar results were obtanied when F_1 injected mice were tested against an allogeneic target (data not shown).

Failure of in vivo primed parental cells to induce GvH-associated immunodeficiency

BR mice were injected weekly i.p. with 10^7 B10 spleen cells. One week following the third injection, 4×10^7 BR spleen cells were injected i.v. into (B10 × BR) F_1 or (BR × D2) F_1 mice and the F_1 CTL response was tested 2 weeks later against TNP-self or allogeneic cells. The CTL response of in vivo primed BR spleen cells was specifically increased (data not shown), but these cells were unable to induce a GvH-associated CTL suppression in (B10 × BR) F_1 (Fig. 2 A). In contrast, CTL suppression was induced when immunized BR spleen cells were injected into (BR × D2) F_1 (Fig. 2 B) demonstrating the specificity of this finding.

Discussion

Numerous experimental models of GvH reactions giving rise to a variety of pathological symptoms have been described and it is not surprising that various effector cells have been reported to mediate the reaction. There is general agreement that mature T cells [9] are the main cause of the disease, but which particular T cells, in terms of phenotype and function, account for the various symptoms is unclear. It was assumed for a number of years that the surface antigen phenotype of lymphocytes defined their functional activity [1, 2]. However, more recently it has been proposed that these phenotypes define the MHC antigen class for which the relevant T cell is specific or restricted, regardless of function [18]. Finally there are several reports supporting the view that there is not an absolute correlation between the phenotype and MHC antigen class specificity [12]. These findings may in part explain the controversy concerning the effector cells when using negative selection of donor T cells in various MHC disparate combinations.

A more direct approach to the question of the effector cells is to use T cell clones known to be $CD4^+$ or $CD8^+$ [13, 19]. However, this alternative method runs the risk that isolated cells no longer represent their ancestor, and the culture itself can select some parental T cell subsets. The functional properties of T cell clones can also be altered compared with those of their ancestor. For example, T cell clones rarely function following an i.v. injection, perhaps because they are no longer able to circulate or can only do so for a limited period of time [14]. Homing studies have shown that the majority of the injected T cell clones reside in lung and liver [19]. Furthermore, GvH disease induced by T cell clones differs in several respects form the disease caused by parental cells [19].

Because of these conceptual and technical pitfalls, we chose a functional approach to determine whether GvH-associated immunosuppression observed across a whole H-2 difference was correlated with parental cytotoxic T cell activity. Parental CTL generated in vitro were unable to induce GvH-associated CTL suppression. Although these cells had been cultured for only 5 days, the validity of this approach could be criticized; we used in vivo priming to confirm the absence of correlation between CTL activity of parental spleen cells and GvH-associated immunodeficiency. Suppressive GvH reaction across a whole H-2 difference is induced by unseparated

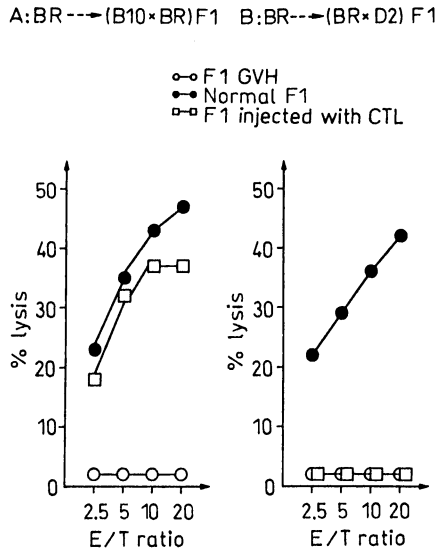

Fig. 2. Inability of in vivo generated CTL to induce GVH-associated immunosuppression. BR CTL were generated by repeated i.p. immunization with B10 spleen cells and injected into **A** (B10 × BR) F_1 or **B** (BR × D2) F_1. CTL assays were performed 2 weeks later against TNP-self

donor T cells, and it seems that $CD4^+$ and $CD8^+$ cells are both necessary in non-irradiated animals [14, 15]. Opinion is divided, however, on the cytotoxic or suppressor function of the $CD8^+$ cells [14, 15]. There are no convincing reports supporting the theory that CTL could easily could detectable in non-irradiated F_1 hybrid recipients undergoing a GvH reaction, and lethal GvH disease can develop in response to MHC antigens in the absence of CTL activity [5, 7]. In irradiated F_1 animals, the role of $CD8^+$ cells remains controversial and the relative contribution of various T cell subsets is determined by the particular strain combination and conditioning regimen [3, 8, 16]. In our model, the functional data which we reported, seemed to confirm the finding that parental CTL are probably not involved in GvH-associated CTL suppression. The GvH-associated CTL suppression observed in a third-party hybrid suggested that the inability of alloactivated T cells to induce immunodeficiency was not merely related to the release of various cytokines.

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