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Role of CTLA4-Ig on induction of unresponsiveness to multiple minor alloantigens

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Abstract LPS blasts inhibited graft rejection in multiple minor incompatible strain combinations but not in presensitized animals or in major histoincompatible combinations.

Key words Tolerance · CD28: B7

Introduction

It is now believed that T cells become activated for allorecognition after encountering antigen-MHC signals, as well as so-called "second signals", most likely delivered by accessory molecules on the cell surface. In the absence of such second signals, a state of anergy in the T-cell results [1]. We hypothesized that the state of unresponsiveness induced to multiple minor alloantigens by hepatic antigen-presenting cells (APC) is a result of the failure of these latter cells to provide a second signal involving LFA-1: ICAM-1 interactions [2].

Recent data have suggested that an alternative receptor: co-receptor interaction may be critically important in T cell activation, the CD28: B7 interaction [3]. This interaction can, in turn, be blocked using soluble fusion protein, which interacts with B7 (on APC), the CTLA4-Ig fusion protein [4]. We report here that, even in the presence of CTLA4-Ig, hyporesponsiveness induced by LPS-B cell blasts to multiple minor antigens is unperturbed, even though the B7 antigen is upregulated on such B cell blasts [3, 4].

Materials and methods

All experimental protocols are described in detail elsewhere [2]. Animals received adherent-cell-depleted, anti-thy 1.2 treated B cells, or 72-h LPS B cell blasts, 36 h before skin grafting. Cells were injected intravenously. Mice were either followed daily to assess skin graft rejection, or, in separate studies, were sacrificed at 10 days post grafting as spleen cell donors for lymphocyte responses in vitro. Lymphokine assays (IL-2 or IL-4) were performed using 60-h culture supernatants and with specific target cells (CTLL-2 or CT4.S, respectively) along with the appropriate blocking monoclonal antibodies (IIBII, anti-IL-4;S4-B6, anti-IL-2). These assays are known to detect 1 unit of lymphokine, as defined using recombinant commercial standard preparations [2].

Results

Data in Table 1 show the results of B10.BR skin graft survival in naive or B10.BR immune C3H (multiple minor incompatible) or C57Bl/6 (major histocompatibility difference) mice, with or without pretreatment with resting or LPS-activated B cell blasts. It was clear that resting B cells produced no changes in B10.BR skin graft survival relative to untreated mice (compare rows 1 and 2 in Table 1). However, after preimmunization intravenously with LPS-activated B10.BR B cell, naive C3H mice showed prolonged survival of B10.BR grafts, while C57Bl/6 mice showed decreased survival. Again, there was no modification of graft survival in immune mice. No change in graft-survival third-party grafts (BALB.K) was seen in any group (data not shown).

Thus, despite the fact that LPS-activated B cells expressed increased amounts of B7, the coreceptor for CD28, these cells preferentially downregulated responses

Table 1 B10.BR skin graft rejection after pretreatment with B10.BR-LPS-B cell blasts (*n.t.* not tested)

Pretreatment of mice[a]	Mean standard time rejection (days)[b]			
	Naive		B10.BR immun	
	C3H/HeJ	C57BL/6	CeH/HeJ	C57BL/6
None	14±2.2	13±2.6	7.8±1.5	6.9±2.1
Resting B10.BR B cells	14±2.9	13±3.1	n.t.	n.t.
LPS-activated B10.BR B-cells	19±3.4*	9.1±1.7*	8.1±1.8	7.6±1.5

* $P < 0.05$, Mann-Whitney U test

[a] Groups of eight mice received injections of $5X10^6$ B10.BR-B cells (activated with LPS or resting cells) 36 h before grafting with B10.BR skin

[b] Arithmetic mean (±SEM) skin graft survival in naive mice, or mice grafted 28 days after rejection of a B10.BR skin graft

Table 2 CTLA4.Ig did not inhibit in vitro induction of hyporesponsiveness to multiple minor antigens by LPS-B cells

Pretreatment of responder cells[a]	Immune responses after restimulation[b]		
	3HTdR incorporation	IL-2	IL-4
C3H/HeJ			
None	7635	7.5±2.0	2.3±0.5
B10.BR LPS-B cells	3100*	2.7±0.6*	3.5±0.7*
B10.BR LPS-B cells + CTLA4-Ig	2805*	1.9±0.7*	3.7±1.1
C57BL/6			
None	9865	9.6±2.2	2.1±0.5
B10.BR LPS-B cells	14905*	14 ±3.0*	3.0±1.4
B10.BR LPS-B cells + CTLA4-Ig	7240*	7.6±2.8	2.2±1.4

* $P < 0.05$

[a] $20X10^6$ C3H/HeJ or C57Bl/6 spleen responcer cells were incubated alone, or with $7.5X10^6$ B10.BR LPS-B blasts for 5 days, with/without CTLA4-Ig (10 μg/ml). Cells were washed X2 and $5X10^5$ cells were incubated in triplicate with $5X10^5$ 2000R B10.BR spleen cells. ^{3}HTdR incorporation (Cpm) or cytokines (units/culture) were assessed as in Materials and methods

[b] Arithmetic mean (±SEM) of triplicate determinations

to multiple minor antigens, while leading to (the expected) sensitization for major histoincompatible grafts (C57Bl/6). Similar data have recently been reported by Fuchs and Matzinger [5]. These data are also reminiscent of studies were reported, suggesting that unresponsiveness induced to multiple minor alloantigens after portal venous immunization is associated with changes in lymphokine production from pretreated cells (towards increased IL-4 production) [2]. Accordingly, we next investigated the lymphokine production potential of cells pretreated with LPS-B cell blasts in vitro, and the effect of an inhibotor of the CD28: B7 interaction, CTLA 4-Ig, on that lymphokine production. These data are shown in Table 2.

The data in Table 2 support the results described in Table 1. In naive C3H/HeJ lymphocytes, preincubation with B10.BR LPS-B blasts led to reduced proliferation after reexposure to B10.BR antigen, decreased IL-2 production, and increased IL-4 production. In contrast, when C57Bl/6 lymphocytes were pretreated in this way, enhanced proliferation was seen on reexposure to B10.BR spleen cells, along with increased production of IL-2, i.e., a priming response. Moreover, while CTLA4-Ig blocked this latter priming effect in the major histoincompatibility model (last row of Table 2), this molecule had no noticeable effect in the multiple minor incompatibility mode (3rd row of Table 2).

Discussion

It is known that T cells need multiple triggering signals to become activated for IL-2 production and concomitant graft rejection [1, 3]. We have argued that hepatic APC lack one important ligand: counter ligand pair involved in the delivery of such activation signals, and as a result, antigen presented by hepatic APC is tolerogenic and preferentially induces IL-4 production [2]. It has been suggested that an alternative receptor: counter-receptor pair involved in T cell activation for IL-2 production is

the CD28: B7 pair [3]. The role of this interaction can be studied using CTLA4-lg to block CD28: B7 binding. As reported elsewhere [6], this molecule does indeed inhibit the sensitization (and enhances IL-2 production) seen when major histoincompatible cells are cultured together using, as sensitizing antigen, B cell blasts, which are high expressors of B7 antigens [3]. As expected also, LPS-B blasts sensitized in vitro for enhanced rejection to major histoincompatible cells (Table 1). However, LPS-B cell blasts induced hypo-responsiveness to multiple minor alloantigens (Tables 1 and 2), and, in a fashion analogous to hepatic APC, simultaneously biased T cells to IL-4 production (Table 2 and [2]). This phenomenon, in turn, was apparently independent of CD28: B7 interactions, as judged by the effect of CTLA4-lg (Table 2). A similar finding was recently made in the H–Y system (single minor antigen difference) for LPS-B blasts [5]. Again of interest, this unresponsiveness induced by LPS-B blasts was not seen when immune T cells were used (e.g. Table 1).

These data suggested that naive and immune T cells have different activation requirements that also differ for cells recognizing "processed antigen" (minor histoincompatible cells) as opposed to major histoincompatible cells, "direct recognition". Moreover, they suggested that CD28: B7 interactions are not relevant to the unresponsiveness induced by LPS- B blasts, which leads both to graft prolongation in vivo and decreased IL-2 production in vitro.

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