REGULATION OF LYMPHOKINE PRODUCTION IN EXPERIMENTAL AND HUMAN CHAGAS' DISEASE

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INTRODUCTION

Lymphokines are the major communication molecules of the immune system and as such are essential in the control of the activation and deactivation of immune responses. Lymphokine production, therefore is a good scale by which the immune response potential of animals can be measured. During infection with the protozoan parasite Trypanosoma cruzi, mice experience a profound immunosuppression which extends to their ability to produce interleukin 2 (IL-2), a lymphokine with T cell growth factor activity. Administration of IL-2 either in vitro (1) or in vivo (2) results in the partial recovery of immune responses suppressed by T. cruzi infection. Although most prevalent in the acute phase of T. cruzi infection, immunosuppression extends well into the chronic phase of the infection in mice and possibly man, and effects the ability of the infected host to respond to parasite antigens as well as non-parasite antigens and mitogens (3). Thus a better understanding of the extent of and mechanisms responsible for regulation of lymphokine production in this infection could lead to the generation of protocols which enhance the anti-parasite response and/or increase resistance to infection or disease.

SUPPRESSION OF IL-2 PRODUCTION

As we (1,4,5) and others (6,7) have previously reported, the production of IL-2 by spleen cells in response to stimulation with the T cell mitogen concanavalin A is suppressed during the acute phase of T. cruzi infection. Spleen cells from C57BL/6 mice chronically infected with the Brazil strain of T. cruzi recover the ability to produce normal or near normal levels of IL-2 production as early as day 80 of infection or as late as day 150. More importantly, the production of IL-2 in response to molecules which interact with the T cell receptor complex in either an antigen non-specific fashion (anti-T3 monoclonal antibodies) or antigen specifically (T. cruzi soluble antigen) is also suppressed during the acute phase of infection (Table 1). Parasite antigen-specific IL-2 production is not evident until well after the peak of parasitemia which occurs at approximately 35 days of infection in this model.

Analysis of IL-2 production by peripheral blood mononuclear cells from a limited number of chronic chagasic patients also suggested a relationship between the length of the infection (as inferred from antibody titers and/or presence of anti-parasite IgM) and IL-2 production (8). The level of IL-2 produced by cells from chagasic patients was negatively correlated with anti-T. cruzi antibody titers. Since the actual length of the infection was not known in these cases, it is also possible that depressed IL-2 production in human Chagas' disease may correlate with other factors, including severity of the infection.

TABLE I. Production of IL-2 by spleen cells from mice infected with 10^3 blood-form trypomastigotes of T. cruzi in reponse to different stimuli.

Day of Infection	Units/ml IL-2		
	Stimulus: con A (5ug/ml)	anti-T3 (10 ul/ml)	T. cruzi sonicate (10ug/ml)
uninfected	90.0	17.5	<0.5
14	82.5	0.9	<0.5
35	16.8	0.8	<0.5
55	16.0	2.0	0.9
95	105.0	7.5	1.8

MECHANISMS FOR SUPPRESSION OF IL-2 PRODUCTION

The documentation of suppressed IL-2 production in T. cruzi infection, although providing an explanation for the depressed T cell responses in infected hosts, brings us no closer to understanding the molecular or biochemical basis of immunosuppression in this system. Studies focusing on the mechanisms regulating IL-2 production in murine T. cruzi infections have revealed at least two components, the presence of suppressor T cells bearing the Lyt2 marker and capable of suppressing IL-2 production by normal T cells, and an inherent failure of T cells from infected mice to respond to normal stimuli with the production of IL-2 (4,5).

The evidence for the inherent inability of T cells from infected mice to respond to mitogens with the production of IL-2 comes from two experimental results. Firstly, although freshly isolated spleen cells from acutely infected mice respond poorly to mitogen (or antigen, Table 1), these cells make large amounts of IL-2 in response to the combined stimulation with calcium ionophore and a phorbol ester (PMA, Table 2). Secondly, spleen cells which are rested for 2-3 days in culture prior to stimulation for IL-2 production, make elevated amounts of IL-2 (Table 2). The presence of parasite antigen (in this case 5×10^6 formalin-fixed trypomastigotes) in the pre-culture stage prevents the recovery of IL-2 production. These results suggest that freshly isolated T cells from mice with acute T. cruzi infections have the ability to produce IL-2 but fail to perhaps because either they lack the receptors for stimulation of lymphokine production or fail to transduce the signal to the interior of the cell once bound to the appropriate receptors, defects which can be bypassed by activation with ionophore and PMA.

TABLE 2. IL-2 production is elicited by the combination of PMA and ionophore or by Con A following a pre-culture period.

Responding cells	Stimulus	IL-2 (U/ml)
Experiment 1		
Normal spleen	Con A (5 ug/ml)	162
Day 30 inf. spleen	"	2
Normal spleen	PMA (10 nM) and Ionomycin (1 uM)	349
Day 30 inf. spleen	"	291
Experiment 2		
Normal spleen	Con A (5 ug/ml)	61
Day 26 inf. spleen	"	9
Normal spleen	48 hr pre-culture then Con A	146
Day 26 inf. spleen	"	52
Normal spleen	48 hr w/Ag, then Con A	137
Day 26 inf. spleen	"	6

TABLE 3. Percent positive cells and mean fluorescence intensity of spleen cells stained with anti-Thy 1.2 or anti-T3 monoclonal antibodies

	Percent Positive Cells (Mean Fluorescence Intensity)	
Day of Infection	Anti-Thy 1.2	Anti-T3
uninfected	30.5 (101)	32.1 (99)
14	21.8 (116)	17.0 (78)
30	35.8 (110)	21.4 (68)
47	40.1 (118)	20.8 (70)
120	29.8 (120)	18.4 (74)

Of preeminent importance as a receptor for activating T cells is the T cell receptor complex (TCR) which functions to bind antigen and self MHC, and transduce to the cytoplasm the signal for activation of T cells and lymphokine production by T_h cells. We have examined the possibility that variations in the expression of T cell receptors is in part to blame for the depressed lymphokine production by T cells from T. cruzi infected mice. During the period of maximal suppression of IL-2 production, fewer Thy 1 positive cells from T. cruzi infected mice express T3 (a component of the TCR complex) and those T cells with T3 display fewer molecules than T cells

from normal mice (reflected in lower mean fluorescence intensity, Table 3). In addition, suppressed IL-2 production (Table 1) and proliferative responses (not shown) of T cells from infected mice to anti-T3 monoclonal antibodies confirms that the decrease in T3 expression coincides with decreased biological function of these cells.

CONCLUSIONS AND HYPOTHESES

To date we have focused our attention on regulation of IL-2 production, not only because this lymphokine is necessary for the expansion of many T cell dependent immune responses, but also because it is one of the better characterized lymphokines and is relatively easy to measure. Evidence from other systems suggest that production of some (but certainly not all) lymphokines is coordinately regulated (9). If this is the case, these data on IL-2 production in T. cruzi infected hosts may give us a preview of what to expect when we examine the production of other lymphokines in this system.

We propose that both of the mechanisms discussed above for suppression of IL-2 production in T. cruzi infected mice are driven by the constant presence of parasite antigen in the system. The presence of large amounts of antigen for an extended period of time is known to induce T suppressor cell activation and antigen activation is known to decrease expression of TCR on T cells. The presence of anti-parasite immune responses evidenced by antibody production and the ability of many hosts to limit parasite growth, suggests that suppressed lymphokine production in vivo is not as complete as our in vitro studies might suggest. Thus, it is possible that the observed suppression of lymphokine production in vitro is due in part to the over-stimulation of T cells in vivo, leading to their inability to respond to additional stimulation. Polyclonal activation could also contribute to this apparent exhaustion of the lymphokine producing capacity of T cells from T. cruzi-infected mice. Suppressor cells may act to prevent further depletion of immune capacity by regulating both the antigen-specific and the polyclonal activation of T cells.

The more important questions to focus on now with respect to lymphokine production are 1) Which lymphokines are important in stimulating an effective anti-T. cruzi immune response, 2) Are these lymphokines being produced in vivo and if not, why, and 3) How can we stimulate the immune system to make the appropriate lymphokines which will yield a protective response?

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