Lyt-23$^+$ CYCLOPHOSPHAMIDE-SENSITIVE T CELLS REGULATE THE ACTIVITY OF AN INTERLEUKIN 2 INHIBITOR IN VIVO*

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Recently, some of the rules governing the in vitro activation of murine cytotoxic T-lymphocytes (CTL)[1] have become unravelled (reviewed in 1, 2). Accordingly, a cascade of cell-cell interactions results in the activation of antigen-specific CTL-precursors (CTL-P) (3–6). The realization that biological mediators derived from functionally distinct cell subsets involved in this process can be assayed separately from their producer cells (7–13) has resulted in the Interleukin[2] concept (2, 5) as depicted in Fig. 1. Its salient feature is the observation that T cells require two signals for activation. Signal 1, i.e., antigen binding by clonally distributed antigen receptors, does not represent an inductive signal, but renders the antigen-selected T cells sensitive to the inductive signal 2 provided by Interleukins. Interleukins bind to nonclonally distributed receptors on sensitive T cells, thereby initiating T cell triggering. Accordingly, the Lyt-1$^+$ helper T cell-derived Interleukin 2 (Il-2; formerly T cell growth factor [TCGF]) represents the inductive signal 2 for CTL-P (5, 12, 15, 16), whereas the antigen-presenting cell (APC)-derived Interleukin 1 (Il-1; formerly lymphocyte-activating factor [LAF]) represents the inductive signal 2 for T helper cells (5, 10, 11). There is also evidence that the release of Il-1 from APC is, in turn, controlled by a mediator derived from inducer T cells (17).

Since the Interleukin concept is entirely based on results obtained in vitro, little is known about its relevance in vivo. Provided that it applies also in vivo, and assuming that the nonspecific and nonrestricted Il-2 represents the inductive signal 2 for the in vivo triggering process of clonally derived primary and secondary CTL-P, how then is the specificity of CTL immune reactions maintained? What are the mechanisms that control the nonspecific activity of Il-2 in vivo, and possibly limit its activity to close vicinity of the producer cell, thereby retaining the specificity of CTL induction?

In an attempt to answer these questions, we searched for the presence of a putative Il-2 inhibitor in sera of normal mice. Here we describe the results obtained.

Materials and Methods

Mice. CBA/Ca, BALB/c, and C57BL/6 mice were obtained from OLAC Ltd., Shaw's Farm, Blackthorn, England. The breeding stock of the C57BL/6-congenic strain C57BL/6

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[1] *Abbreviations used in this paper:* APC, antigen-presenting cell; ConA; concanavalin A; CTL, cytotoxic T lymphocytes; CTL-P, cytotoxic T lymphocyte precursors; FCS, fetal calf serum; GVH, graft vs. host; Il-1, Interleukin 1; Il-2, Interleukin 2; K_{av}, average association constant; NMS, normal mouse serum; PBS, phosphate buffered saline; PNA, peanut agglutinin.

[2] At the Second International Workshop, 1979, Ermatingen, Switzerland, a system of nomenclature was introduced to term factors acting as communication signals between leukocytes (14).

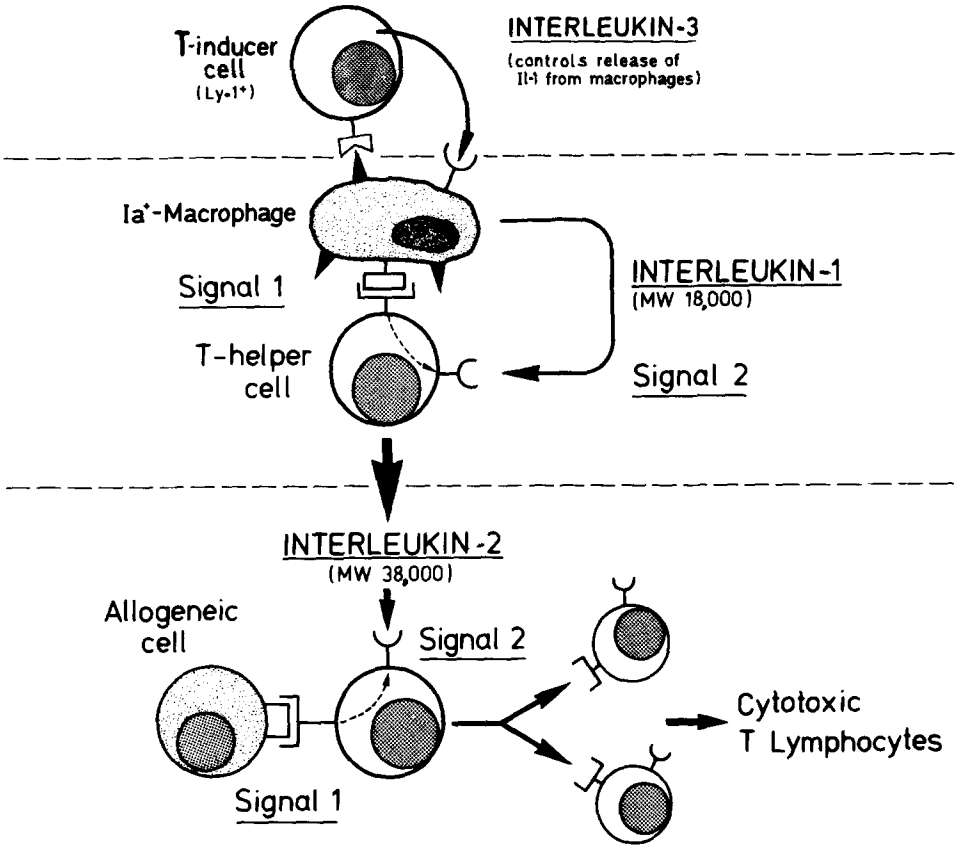

FIG. 1. Minimal requirements of T-T cell interactions during the induction of CTL. The Interleukin concept: - - - → indicates that antigen-binding (signal 1) renders the T cell sensitive to the inductive signal of Interleukin (signal 2). MW, molecular weight.

Lyt^a/Boy (B6/Lyt 1.1) was kindly provided by Dr. E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York. The C3H nu/nu mice were bred in the Max Planck Institute, Freiburg, Federal Republic of Germany. Wistar rats were bred in our own animal facilities at the Johannes Gutenberg University, Mainz, Federal Republic of Germany.

Treatment of Mice. Mice were x-irradiated with 950 rad (Philips RT 200; Fa. Müller, Frankfurt, Federal Republic of Germany) with a dose rate of 50 rad/min (whole-body irradiation). Mice were injected intraperitoneally with 60 mg/kg cyclophosphamide (Endoxan; ASTA-Werke, Bielefeld, Federal Republic of Germany).

Semipurified Interleukin-2 (Il-2). Il-2 was prepared from concanavalin A (Con A)-stimulated lymphocyte culture supernate essentially as described (7). In short, spleen cells (7×10^6/ml) from mice or rats were cultured for 20–24 h in medium without fetal calf serum (FCS) in the presence of 1 μg Con A/ml or 5 μg Con A/ml (Pharmacia Fine Chemicals, Uppsala, Sweden), respectively. The supernate was concentrated by ultrafiltration with an Amicon YM-10 membrane (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) and the concentrate applied to a previously calibrated Sephadex G-100 column. Effluent fractions were tested for their capacity to sustain growth of long-term CTL in vitro (7). Active fractions were pooled, concentrated, and kept frozen at −20°C.

Il-2 Inhibitor. The source of Il-2 inhibitor was serum of normal inbred mice or inbred rats. Serum was obtained by bleeding ether-anesthetized animals from the retroorbital sinus or by cardiac puncture. Serum was filter-sterilized through 0, 2 μm Millipore filters (Millipore Corp., Bedford, Mass.) and used immediately or stored at 4°C.

Ammonium Sulfate Precipitation. Ammonium sulfate solution was added to fresh normal mouse serum (NMS) to 50% saturation. The mixture was allowed to stand overnight at 4°C. After centrifugation, the supernate was dialyzed extensively against 0.02 M phosphate-buffered saline (PBS, pH 7.4) and concentrated with an Amicon YM-10 ultrafiltration membrane.

Molecular Weight Estimation. Chromatography and molecular weight estimation was done according to the Pharmacia Fine Chemicals Inc. instruction manual. Gel filtration was performed at 4°C with Sephadex G-200 equilibrated with 0.02 M PBS (bed dimensions 1.76 × 100 cm). The sample vol was 1 ml and 2-ml fractions were collected. The column was calibrated with marker proteins of known molecular weight (bovine serum albumin, ovalbumin, and ribonuclease; gel filtration calibration kit; Pharmacia Fine Chemicals Inc.) and their elution positions monitored by ultraviolet ($A_{280\ nm}$) absorbance.

The average association constant (K_{av}) values of each protein were calculated using the equation:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0},$$

where V_e is the elution volume for the protein, V_0 is the column void volume determined from the elution volume of blue dextran 2000. Using semi-logarithmic graph paper, the K_{av} value for each protein was plotted (linear scale) against the corresponding molecular weight (logarithmic scale).

1 ml of nonprecipitable serum constituents [at 50% concentration of $(NH_4)_2SO_4$] was applied to the column and effluent fractions were dialyzed against medium. The elution position of Il-2 inhibitor was determined by testing samples of fractions for their capacity to functionally inactivate the effect of Il-2 on secondary cytotoxic T lymphocytes (7). The K_{av} values were calculated and the molecular weights determined from the calibration curve of marker proteins.

Antisera. Monoclonal anti-Lyt-1.1 antibodies were kindly provided by Dr. I. F. C. McKenzie (Austin Hospital, Heidelberg, Victoria, Australia) and monoclonal Lyt-2.2 antibodies by Dr. U. Hämmerling (Memorial Sloan-Kettering Cancer Center). Monoclonal anti-Thy-1.2 antiserum was kindly provided by Dr. P. Lake (University College, London). Rabbit anti-mouse Ig antibodies were obtained after immunization with purified mouse Ig. Before use, the antiserum was extensively absorbed with mouse thymocytes; thereafter, the titer was 1:400. The use of anti-Lyt antisera has been described elsewhere (7). Briefly, cells were resuspended in the diluted antiserum at a concentration of 10^7 cells/ml and incubated for 30 min at 4°C, centrifuged, resuspended in selected nontoxic rabbit complement diluted 1:12, and incubated for 45 min at 37°C. The number of viable cells remaining after treatment was determined by the dye exclusion method. Dead cells were removed by centrifugation over Ficoll.

Purification of T and B Cells. T cells were purified by passage of spleen cells over a nylon wool column as described (18), B cells were obtained after treatment of spleen cells with anti-Thy-1 serum and complement.

Positive Selection of Lyt-123$^+$ Cells. As already described (16) virtually all peanut agglutinin (PNA)-binding thymocytes express the Lyt-123$^+$ phenotype. To positively select Lyt-123$^+$ cells, PNA-thymocytes were purified by cell-affinity chromatography according to the method of Irlé et al. (19) as described (16). PNA-binding thymocytes were recovered by washing the column with medium containing 0.15 M D(+)galactose. The PNA$^+$ cells (>99% PNA$^+$ in direct immunofluorescence) proved to be all Lyt-123 positive, as tested in a complement-dependent cytotoxicity assay with the appropriate anti-Lyt antisera.

Cell Culture and Assay of CTL

CULTURE MEDIUM. A mixture of Click's and RPMI-1640 media (50% vol:vol) was supplemented with 10 mM Hepes, fresh glutamine, 5×10^{-5} M 2-mercaptoethanol, and 5% FCS.

SECONDARY MIXED LYMPHOCYTE CULTURES. 3.5×10^6 spleen cells were cultured with 1.5×10^6 x-irradiated stimulator cells (2,000 rad, dose rate of 620 rad/min; Philips RT 200) in 2 ml medium in multiculture plates (Linbro FB-24; TC; Linbro Chemical Co., Hamden, Conn.) in a humified atmosphere of 5% CO_2 in air. At day six, 5×10^5 viable cells were restimulated with 2.5×10^6 x-irradiated stimulator cells. After 9 d of culture, cells were harvested and centrifuged over Ficoll to remove dead cells.

TEST FOR Il-2 INHIBITOR ACTIVITY. 2×10^4 secondary CTL were cultured in the presence of an optimal concentration of Il-2. Il-2 inhibitor, i.e., serum to be tested for inhibitor activity, was added at the initiation of culture. At day 3, the CTL generated were tested for cytotoxic activity.

TARGET CELLS. P815 ($H\text{-}2^d$) and EL_4 ($H\text{-}2^b$) tumor cells were propagated in vitro.

CYTOTOXICITY ASSAY. Graded numbers of viable cells were harvested from mixed lymphocyte cultures and were incubated for 3 h with a constant number (5,000) ^{51}Cr-labeled target cells as described elsewhere (13). The percent specific lysis was calculated according to the formula described previously (13).

Results

Il-2 Inhibitor in the NMS. The Lyt-1^+ helper T cell-derived Il-2 is functionally defined by its capacity to induce and to sustain clonal expansion of Il-2-sensitive T-cells (1, 2, 8, 20, 21). In the present studies as test system for the putative Il-2 inhibitor the inhibition of Il-2 driven, clonal expansion of alloreactive CTL was used. The results depicted in Fig. 2 show that sera of adult mice contain high Il-2 inhibitory activity, the activity of which, in turn, can be overcome by increasing concentrations of Il-2 (Fig. 3). Most interesting was that unlike NMS, the sera of athymic (nu/nu) mice were found to be devoid of Il-2 inhibitory activity (Fig. 2, 3). In fact, sera of nu/nu mice supported expansion of alloreactive CTL equally well as FCS (Figs. 2, 3). These results provided circumstantial evidence that the Il-2 inhibitory activity in NMS is dependent on an intact T cell system. Il-2 inhibitory activity was absent in amniotic fluid and in sera taken from mouse embryos (Fig. 4). Within 7 d after birth, the serum concentration of Il-2 Inhibitor activity reached the level found in adult mice (Fig. 4). Sera of aged mice appear to have a reduced Il-2 inhibitory activity (Fig. 4). Preliminary results indicated that sera of adult NZB mice prone to undergo autoimmune reactions exhibit only ~10–20% of Il-2 inhibitory activity as compared with normal mice (C. Hardt, unpublished data).

Preliminary experiments have shown that Il-2 inhibitor was not precipitated by $(NH_4)_2SO_4$ up to 50% saturation. This allowed us to enrich for Il-2 inhibitor by precipitating contaminating serum proteins at a $(NH_4)_2SO_4$ concentration of 50%.

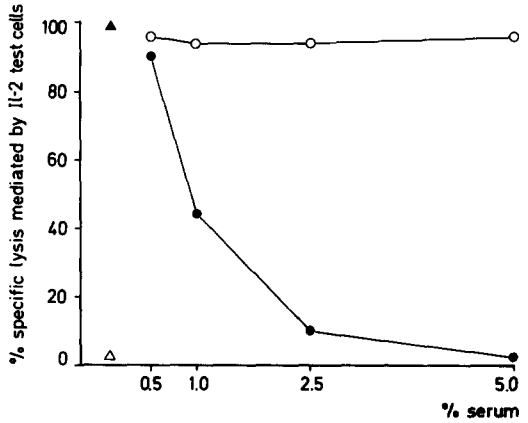

FIG. 2. Dose-dependent, Il-2-inhibitor activity in NMS. 2×10^4 primed CBA anti-BALB/c CTL were cultured together with 50 μl Il-2. Graded concentrations of NMS (●) or sera of athymic nu/nu mice (○) were titrated into the culture and cytolytic activity was determined on day 3. Control cultures received no mouse serum: ▲, positive control (with Il-2); △, negative control (without Il-2).

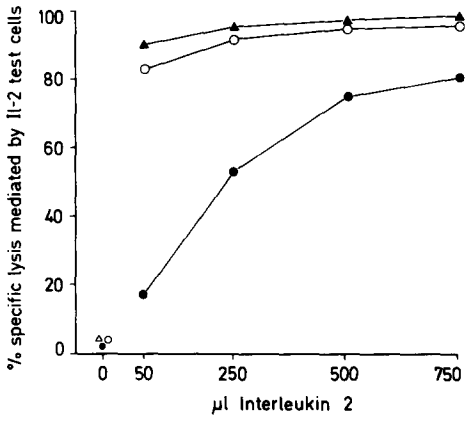

FIG. 3. Increasing amounts of Il-2 can overcome the Il-2-inhibitor activity of NMS. 2×10^4 primed CBA anti-BALB/c CTL were cultured together with (●) 2% NMS; (○) 10% nude mouse serum; or (▲) no mouse serum. Increasing amounts of Il-2 were titrated into the system and cytolytic activity of CTL generated was determined on day 3. No CTL were generated in the absence of Il-2.

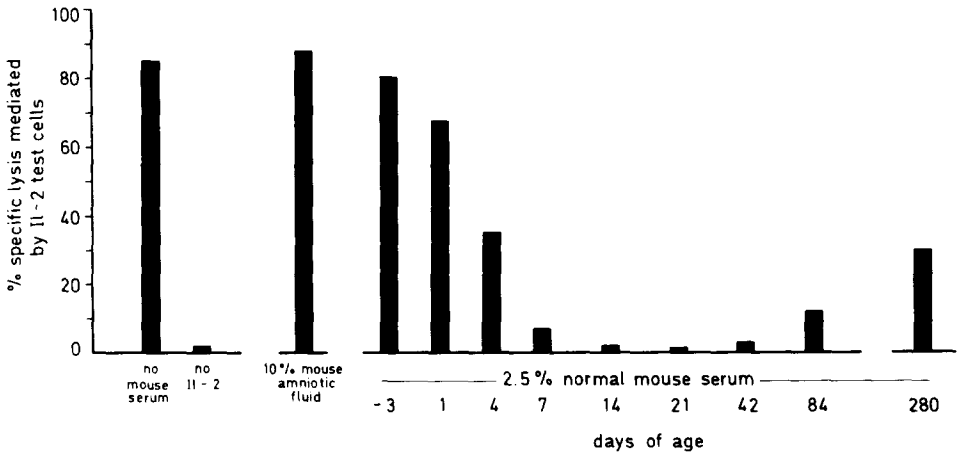

FIG. 4. Age dependency of the presence of Il-2 inhibitor in sera of normal mice. 2×10^4 CBA anti-BALB/c primed T cells were cultured in the presence of 50 µl Il-2. NMS from unborn mice and serum from mice of different ages were added to the cultures at final concentrations of 2.5%. Mouse amniotic fluid was tested at a final concentration of 10%. After 3 d, CTL generated were tested for their cytolytic activity in a 3-h ^{51}Cr-release assay.

Fractionated elution of nonprecipitable serum constituents on Sephadex G-200 allowed estimation of the molecular weight of the Il-2 inhibitor. As shown in Fig. 5, the Il-2 inhibitor eluted at ~50,000 mol wt (Fig. 5). The data given in Table I show that the Il-2 inhibitory activity is not H-2 restricted. In addition, the Il-2 inhibitory activity is not linked to the antigen specificity of the T cell driven by Il-2 into clonal expansion. However, the data presented here do not allow us to determine whether the Il-2 inhibitor neutralizes Il-2 in the fluid phase or after binding to its target cells. Titration of the effect of either rat or mouse Il-2 inhibitor on mouse or rat Il-2 revealed no detectable species specificity (Table II). This finding is in agreement with the finding that mouse Il-2 and rat Il-2, although different with regard to their molecular weights (22), do not exhibit species specificity.

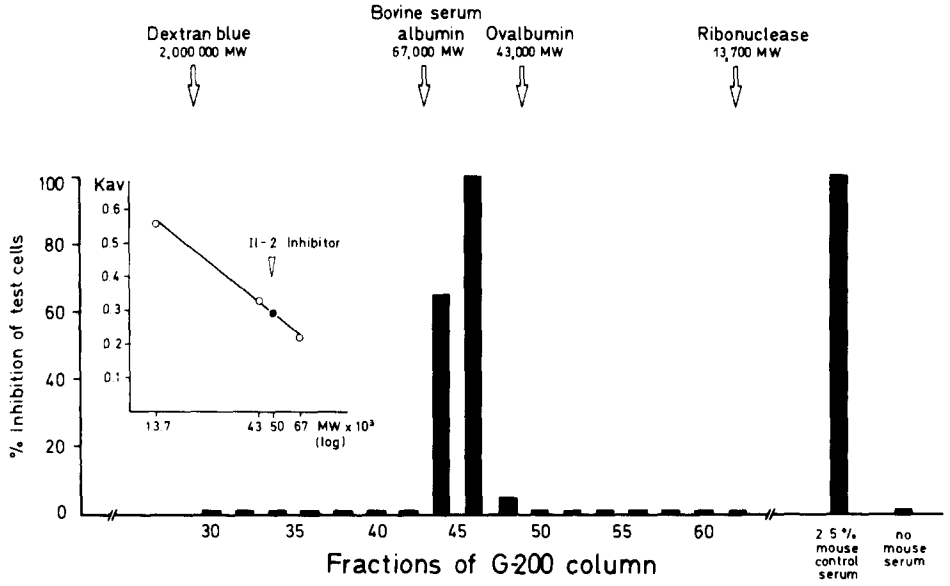

FIG. 5. Molecular weight estimation of Il-2 inhibitor by elution from a Sephadex G-200 column. See Materials and Methods for details. MW, molecular weight.

TABLE I

Nonspecificity of Il-2 Inhibitor

Il-2 test cells	Il-2 inhibitor from normal mice	Percent inhibition in the presence of		
		5% serum	2.5% serum	0.5% serum
CBA anti-BALB/c	CBA	100	48	0
	BALB/c	100	35	0
CBA anti-C57BL/6	CBA	99	48	0
	BALB/c	97	30	0
C57BL/6 anti-BALB/c	CBA	100	40	0
	BALB/c	96	24	0

2×10^4 primed CTL were cultured together with 50 μl Il-2 (derived from $H\text{-}2^d$ spleen cells), 5% FCS, and NMS as indicated. Cytolytic activity of CTL generated was tested on day 3. Percent inhibition is calculated from the reduction of percent specific lysis relative to the control culture—where no serum was added. Percent specific lysis of Il-2 test cells was, depending on the group, between 65 and 94%.

Characteristics of Cells Controlling the In Vivo Activity of Il-2 Inhibitor. As already discussed, sera of athymic (nu/nu) mice lack the Il-2 inhibitor (Figs. 2, 3). In addition, 3–4 d after whole-body irradiation (950 rad) of normal mice, the Il-2 inhibitor disappeared (Fig. 6). Taken together these results suggested that T cells control the in vivo activity of the Il-2 inhibitor and that there exists in vivo a delicate balance between Il-2-inhibitor production and its clearance. To define more precisely the role of T cells for the production of the Il-2 inhibitor, cell transfer experiments into nu/nu mice were performed. The results given in Fig. 7 demonstrate that unlike syngeneic

TABLE II

Lack of Specificity of Interleukin-2 Inhibitor from Mouse and Rat Sera

Il-2 inhibitor	Percent serum	Percent inhibition			
		CTL: CBA (mouse) anti-BALB/c		CTL: Wistar (rat) anti-BALB/c	
		Mouse Il-2	Rat Il-2	Mouse Il-2	Rat Il-2
NMS	20.0	100	100	100	100
	10.0	100	94	100	100
	5.0	99	80	100	100
	2.5	63	42	50	70
	0.5	0	0	9	15
Normal rat serum	20.0	94	75	100	100
	10.0	42	12	59	49
	5.0	0	0	53	42
	2.5	0	0	17	27
	0.5	0	0	0	0

2×10^4 primed T cells from mouse or rat sera were cultured in the presence of 50 μl Il-2 (Con A-induced Il-2 from mouse or rat spleen cells) and 5% FCS. As source of Il-2 inhibitor, NMS or rat serum was added to final concentrations as indicated. Cytolytic activity of CTL was determined on day 3. Percent inhibition is expressed as the reduction of percent specific lysis relative to the cultured cells to which no serum was added. Percent specific lysis of the control culture: A, 83%; B, 93%; C, 61%; D, 64%.

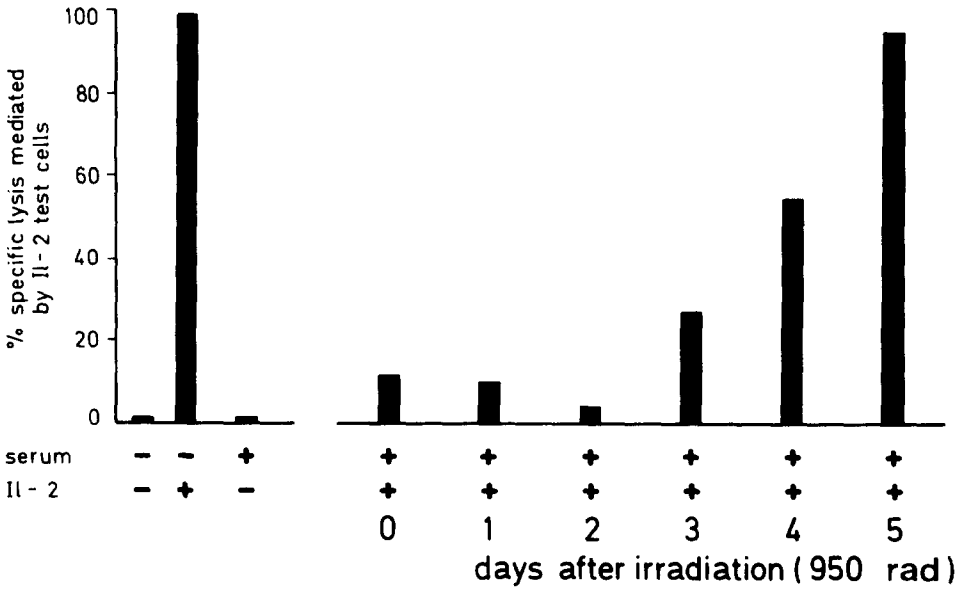

FIG. 6. Effect of whole-body irradiation on the concentration of serum-borne Il-2 inhibitor. 6-wk-old CBA mice were x-irradiated with 950 rad. At the time intervals given, three mice per group were bled from the retroorbital sinus and the serum stored at 4°C. All sera obtained were tested together for inhibitor activity as detailed in Materials and Methods.

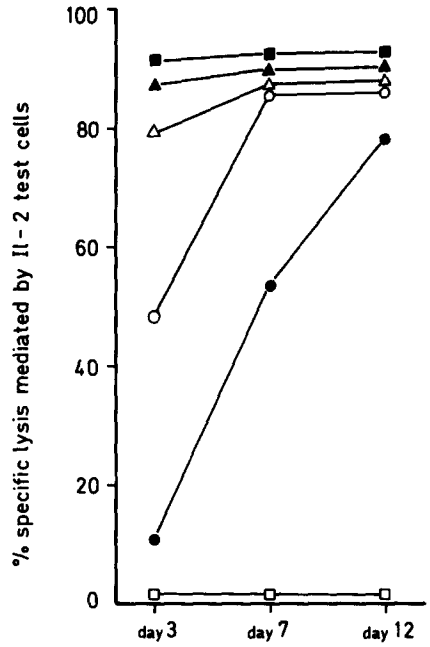

FIG. 7. T cell-dependent induction of Il-2 inhibitor in sera of athymic mice. Groups of nine C3H nu/nu mice were grafted with 3×10^7 cells as depicted. ●, Allogeneic C57BL/6 T cells (>95% T cells); ○, allogeneic C57BL/6 T cells, 2,000-rad irradiated (>95% T cells); ▲, syngeneic C3H T cells (>95% T-cells); △, allogeneic C57BL/6 B cells (>99% B cells); ■, no cells; □, control of normal C3H mouse serum. At the time points indicated, mice were bled and the sera stored at 4°C. Thereafter, the sera were tested for Il-2 inhibitory activity.

T cells, upon transfer of allogeneic T cells, the sera of the recipient nu/nu mice contain high levels of Il-2 inhibitor activity within 3 d. Interestingly, the serum activity of Il-2 inhibitor has almost disappeared 7–12 d after transfer of allogeneic T cells.

Because nu/nu mice are able to reject grafted allogeneic T cells within 7–8 d (23, 24) and because the data depicted in Fig. 6 have suggested an Il-2-inhibitor clearance time from the sera of ~3–5 d, the data given in Fig. 7 provide circumstantial evidence that the Il-2 inhibitor is derived from the grafted allogeneic T cells. Moreover, only the Lyt-23^+ T cell subset, but not Lyt-1^+ T cells, appeared to be endowed with the capacity to induce Il-2 inhibitor activity in the sera of recipient nu/nu mice in a graft-vs.-host (GVH) reaction (Fig. 8). Because pretreatment of normal CBA mice with moderate doses (60 mg/kg) of cyclophosphamide, a protocol previously shown to affect primarily T suppressor cells (25, 26) also resulted in a dramatic decrease of Il-2 inhibitor activity (Fig. 9), we conclude that the Il-2 inhibitor is controlled by a cyclophosphamide-sensitive Lyt-23^+ T cell in vivo.

Discussion

We have identified an Il-2 inhibitor with an ~50,000 mol wt in NMS. Sera of athymic (nu/nu) mice lack the Il-2 inhibitor. Il-2 inhibitor appearance was found to be age related and thoroughly dependent on the reactivity of cyclophosphamide-sensitive Lyt-23^+ T cells. The functional activity of the Il-2 inhibitor appeared to be neither H-2 restricted, nor antigen specific, nor species restricted, at least between

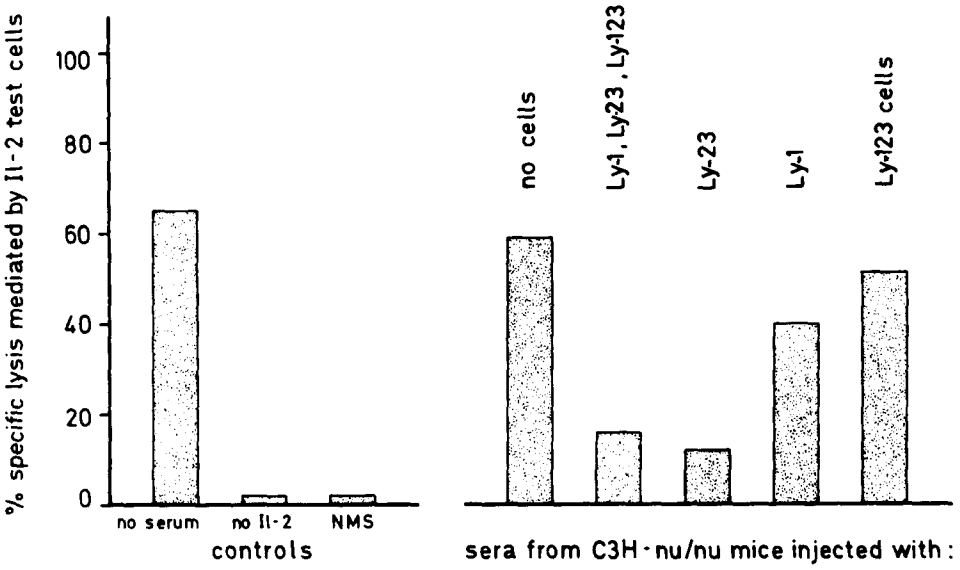

FIG. 8. Ly-1 congenic, nylon wool-nonadherent C57BL/6 spleen cells were treated with anti-Ly 1.1 or anti-Ly 2.2 antiserum and complement or left untreated. As a source of Ly-123$^+$ T cells, PNA$^+$ thymocytes (12) were used. Groups of two individual C3H mice were grafted with either 35 × 10^6 untreated C57BL/6 T cells, 30 × 10^6 PNA$^+$ Ly-123$^+$ thymocytes, 12 × 10^6 Ly-23$^+$ C57BL/6, or 24 × 10^6 Ly-1$^+$ C57BL/6 T cells. 3 d after cell transfer, individual mice were bled, sera were pooled per group, and tested for the presence of Il-2 inhibitor as detailed in Materials and Methods.

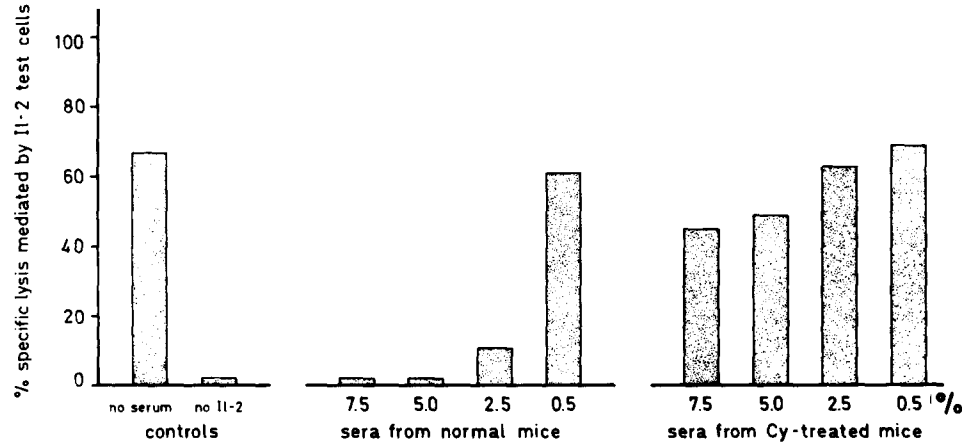

FIG. 9. CBA mice were either injected for 3 consecutive d intraperitoneally with 60 mg/kg cyclophosphamide (Cy) or with an equivalent vol of NaCl. The mice were bled 24 h later, and their sera tested at graded concentrations for the presence of Il-2 inhibitor activity.

mouse and rat. However, further analysis is required to establish a similar pattern of species restriction as described for Il-2 (27) for the Il-2 inhibitor. Because of the recently recognized central role of the Lyt-1$^+$ helper T cell-derived Il-2 as inductive signal 2 (Fig. 1) in the in vitro triggering process of antigen-specific CTL-P (1–9), and because of the nonspecific and unrestricted function of Il-2 (5, 6, 13), we postulated that under in vivo conditions, regulatory mechanisms must exist as a consequence of

which the functional activity of Il-2 is limited to close proximity of its producer cells, thereby avoiding induction of third-party Il-2-sensitive CTL-P.

This report is first to describe high endogenous levels of Il-2 inhibitor activity in the sera of normal mice. Its absence in the amniotic fluid, its rise in the early postnatal period, its ~50,000 mol wt, and its capacity to selectively neutralize Il-2 activity excludes the possibility that we are dealing with alpha-fetoprotein, which is known to induce suppressor T cells (28, 29). The absence of Il-2 inhibitor in athymic nu/nu mice, and its appearance in the course of a GVH reaction mediated by allogeneic Lyt-23$^+$ T cells strongly points out that only in the course of activation of the Lyt-23$^+$ T cell subset, Il-2 inhibitor activity can be detected in the serum of recipient nu/nu mice. It is well known that the Lyt-23$^+$ T cell subset includes effector cells of the T suppressor circuit (30, 31).

The presence of Il-2 inhibitor in sera of normal unprimed mice implies a permanent in vivo stimulation of the Il-2-inhibitor-producer cells. The observation that a cyclophosphamide-sensitive Lyt-23$^+$ T cell is required for its production provides additional circumstantial evidence that the Il-2 inhibitor, in fact, represents a nonspecific effector molecule derived from suppressor T cells.

Although further experimentation is required for a precise analysis of the cell producing the Il-2 inhibitor and of the mode of action of the inhibitor on Il-2 activity, the results discussed here provide compelling evidence for the existence of a cyclophosphamide-sensitive T cell regulatory system able to neutralize in vivo the activity of Il-2. Because in NMS the Il-2 inhibitor activity is high, the functional activity of the helper T cell-derived Il-2 will be restricted, in vivo, to its site of production. It follows then that a close proximity between helper T cells and CTL-P is required for effective in vivo CTL induction to occur.

The presence of high concentrations of Il-2 inhibitor activity in sera of normal mice poses limitations on a protocol aimed at boosting CTL responsiveness toward weakly immunogenic antigens by systemic or local applications of Il-2 in vivo. According to our experience (32), the amplifying effect of exogenous Il-2 on the in vivo CTL responsiveness toward trinitrophenyl-conjugated syngeneic cells was rather poor. Obviously, the prospects of such a protocol could be improved by either pretreatment of the recipient with low doses of cyclophosphamide, a maneuvre known to selectively paralize suppressor T cells (25, 26) and shown here to reduce dramatically the activity of Il-2 inhibitor (Fig. 9).

Alternatively, a good prospect is conceivable in in vivo situations characterized by a lack of Il-2 inhibitor. Indeed, we recently could show a dramatic effect of systemically applied exogenous Il-2 on both the in vivo CTL responsiveness (24) and the helper T cell responsiveness (33) of athymic nu/nu mice in vivo.

The relation of the Il-2 inhibitor described here with immunosuppressive factors described in the literature (34–38) is yet unknown. It is also unclear whether the Il-2 inhibitor described here is related to the nonspecific product of cloned suppressor T cells, as analyzed by Cantor et al. (39). To study these questions there is an obvious need to establish in vitro conditions that allow production of the Il-2 inhibitor from their producer cell. The existence of the Il-2 inhibitor in sera of normal mice may explain the low in vivo primary CTL responsiveness (40–42). The observation that 3–4 d after whole-body irradiation of normal mice, the serum-borne Il-2 inhibitor activity disappears (Fig. 6) might also explain why in the spleen of irradiated (A ×

B)F_1 mice, but not in those of normal (A × B)F_1 mice, highly alloreactive CTL are present, provided the mice have been injected with parental type of responder T cells (43). On the basis of the results described here, we propose that the relative concentration of Il-2 vs. Il-2 inhibitor will decide whether or not the inductive signal 2 for the activation of CTL-P is available in vivo.

Summary

Sera of thymus-bearing normal mice contain high levels of Interleukin 2 (Il-2) inhibitor, whereas sera of athymic nu/nu mice do not. Evidence is presented that cyclophosphamide-sensitive Lyt-23^+ T cells induce high Il-2 inhibitor activity in the recipient nu/nu mice in the course of a graft-vs.-host reaction. The Il-2 inhibitor has an ~50,000 mol wt. Its function is neither antigen specific nor H-2 restricted. During ontogeny, its activity parallels the development of T cell reactivity, i.e., it is absent both in the amniotic fluid and in sera of unborn mice, but increases to high levels during the early postnatal phase.

The Il-2 inhibitor described is viewed as an example of a T cell-dependent, in vivo regulatory mechanism able to effectively counteract the nonspecific activity of the Lyt-1^+ helper T cell-derived Il-2. Because the Il-2 inhibitor activity is rather high in vivo, Il-2 activity will exist only in close proximity to its producer cell, thereby maintaining specificity during the in vivo induction of cytotoxic T lymphocytes.

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