0022-1767/85/1345-3108$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 134, No. 5, May 1985
Printed in U.S.A.

INDUCTION OF INTERLEUKIN 2 RESPONSIVENESS IN THYMOCYTES BY SYNERGISTIC ACTION OF INTERLEUKIN 1 AND INTERLEUKIN 2[1]

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Thymocyte cultures from C3H/HeJ mice were stimulated for proliferative responses with purified preparations of interleukin 1 (IL 1) and interleukin 2 (IL 2). Synergistic responses were obtained in the absence of mitogen. In the presence of excess IL 2, the thymocyte proliferation response was strictly dependent on the amount of IL 1 in the cultures. Antibodies to IL 1 inhibited the response in a dose-dependent manner. The combination of IL 1 plus IL 2 induced the appearance of IL 2 receptors on murine thymocytes as detected with a monoclonal antibody directed against the IL 2 receptor. Neither IL 1 nor IL 2 alone had this effect. The thymic subpopulation found to become IL 2 responsive upon IL 1 stimulus was the peanut agglutinin-negative (PNA^-) medullary fraction.

Interleukin 1 and 2 (IL 1 and IL 2) play important roles in the generation of T and B cell immune reactivities (reviewed in References 1–4). A well-established function of the macrophage product IL 1 is the promotion of IL 2 production from T cells (5, 6). IL 2 appears to be required for the sustained long-term growth of T cells and for the generation of cytotoxic T cell responses (7–9). Both IL 1 and IL 2 have been shown to augment the proliferative response of thymocytes to suboptimal doses of T cell mitogens like concanavalin A (Con A) (10, 11). The thymic subpopulation with capability to proliferate upon mitogen and IL 1 stimulus was found to be the peanut agglutinin-negative (PNA^-)[2] medullary type (12). This stimulation has been shown to result in the release of IL 2 by the PNA^- cells and the subsequent utilization of the IL 2 by the same cell population (13).

The question arose as to whether the induction of IL 2 production is the only function of IL 1. Experiments detailed in this paper suggest that within the PNA^- thymic subpopulation, IL 1 exerts a function in addition to the generation of IL 2. This second function of IL 1 enables PNA^- cells to utilize IL 2 for subsequent proliferation. IL 2 receptor expression seems to be regulated by synergistic action of IL 1 and IL 2 in the thymocyte cultures. PNA-positive (PNA^+) thymocytes in the presence of IL 1 were unable to make use of the IL 2 that was provided. This indicated that IL 1 itself is not directly acting on immature thymocytes to become IL 2 responsive, but rather synergizes with IL 2 to make PNA^- cells responsive to IL 2.

Received for publication September 18, 1984.
Accepted for publication January 2, 1985.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported in part by National Science Foundation Grant PCM-8110370 to S. B. M. and by Deutsche Forschungsgemeinschaft Grant MA 760/3-1 to D. N. M.
[2] Abbreviations used in this paper: PNA, peanut agglutinin; FITC, fluorescein isothiocyanate.

MATERIALS AND METHODS

Mice. Male C3H/HeJ mice were purchased from the Jackson Laboratory, Bar Harbor, ME. All animals were used at 4 to 9 wk of age.

Culture medium. The culture medium used was RPMI 1640 (GIBCO) and was supplemented with 10% heat-inactivated fetal calf serum (FCS), L-glutamine (2×10^{-3} M), HEPES (0.02 M), 2-mercaptoethanol (4×10^{-5} M), and gentamicin (50 μg/ml).

Preparation of IL 2. IL 2 preparations were prepared as described by Hilfiker et al. (14). EL-4 thymoma cells were stimulated in RPMI 1640 containing 0.5% heat-inactivated FCS with phorbol myristic acetate (10 ng/ml). After 48 hr, the cellfree supernatant was concentrated on a Amicon PM-10 membrane; proteins were precipitated with ammonium sulfate (80% saturation) and applied onto a phenyl-Sepharose column. IL 2 was eluted with 40% ethanediol in 0.16 M ammonium sulfate. After concentration of the IL 2-containing fractions on an Amicon PM-10 membrane, the material was applied onto a Sephadex G-100 column. The IL 2 activity eluted with an apparent m.w. of approximately 32,000.

Recombinant human IL 2 was kindly provided by Cetus Corp. The material was 98% pure, containing 0.02 ng/ml endotoxin, and had an IL 2 concentration of 2×10^5 U/ml.

Assay for IL 2 activity. The amount of IL 2 in supernatants was assayed with an IL 2-dependent cell line, W-2 (15). Thymidine incorporation after a 20-hr incubation and a 4-hr pulse with 1.0 μCi of [^{3}H]thymidine (^{3}HTdR; specific activity 50 Ci/mmol; Amersham International Ltd., Amersham, U.K.) was determined. Results are expressed in units as described by Farrar et al. (16).

Preparation of IL 1. IL 1 was obtained from superinduced $P388D_1$ cells and was purified by gel filtration and immunoaffinity chromatography (17).

Preparation of anti-IL 1 antibodies. Anti-IL 1 antibodies were raised in a goat as described (17). The IgG fraction from the heat-inactivated serum was isolated on DEAE cellulose ion-exchange chromatography after ammonium sulfate precipitation. The protein concentration was adjusted to 5 to 10 mg/ml.

Assay for IL 1 activity. IL 1 was measured in the C3H/HeJ mouse thymocyte assay (18). One unit of IL 1 per milliliter induced 50% of the maximal thymocyte proliferative response.

Thymocyte proliferation. Thymocytes (1 to 3×10^5 per culture) from C3H/HeJ mice were cultured in 0.2-ml volumes for 3 days. Varying amounts of either IL 2 and/or IL 1 preparations were added to these cultures. ^{3}HTdR uptake was measured in a 7-hr pulse, and results are expressed in cpm of duplicate or triplicate cultures ± SD.

Separation of PNA^+ and PNA^- thymocytes by fluorescence-activated cell sorting. Thymocytes were separated after staining with subagglutinating concentrations of PNA-FITC[2] (12). PNA-FITC at a concentration of 5 μg lectin/ml was used for staining 10^7 cells/0.25 ml for 30 min at 4°C. The cells were diluted to 5×10^6 cells/ml (without washing) for sorting in a fluorescence-activated cell sorter (Ortho Diagnostic System). Cells in channels 0 through 32 were collected as PNA^- fraction, and in channels 38 through 100 as PNA^+ fraction. After sorting, samples of fractionated thymocytes were routinely analyzed to assess purity. The sorted cell fractions were washed, then resuspended in culture medium; the viable cells were

counted in a hemacytometer.

IL 2 receptor determination. Glutaraldehyde-fixed sheep erythrocytes (5×10^6) were added to triplicate cultures of 4×10^5 thymocytes, and the cells were kept on ice for 30 min in the presence or absence of culture fluid (100 μl) from a rat hybridoma cell line that secretes antibodies directed against the murine IL 2 receptor (AMT 13) (19). The cells were then incubated for an additional 60 min on ice with ^{125}I-labeled F(ab')$_2$ fragments of a sheep anti-rat IgG (100 μl of a 1/10 diluted stock with specific activity of 12 μCi/μg; Amersham International Ltd.). The cells were washed, and specific binding was determined by subtracting nonspecifically bound radiolabel of parallel cultures and was expressed as cpm ± SD.

RESULTS

Induction of thymocyte proliferation. Both IL 1 and IL 2 have been characterized by their capacity to induce synergistic proliferative responses after stimulation with suboptimal amounts of T cell mitogens (10, 11). The IL 1-dependent mitogenesis appears to be due at least in part to the subsequent production, release, and use of IL 2 (6). The results shown in Figure 1 demonstrate that in the absence of mitogen, purified preparations of IL 1 and IL 2 act synergistically to induce thymocyte proliferation. IL 1 alone (up to 0.5 U/culture) induced less than 1000 cpm ^{3}HTdR uptake in cultures with 3×10^5 or 1×10^5 thymocytes. IL 2 preparations that differed in source as well as in IL 2 concentration resulted in identical dose-response curves for the IL 1 effect. In panel *A*, the IL 1-induced mitogenesis in the presence of 10 U partially purified murine IL 2 was compared with the mitogenic response in the presence of 5 U of recombinant human IL 2. The resulting ^{3}HTdR uptake data from all cultures fit a straight line with a correlation coefficient of $r^2 = 0.991$. Similar data were obtained with cultures containing 1×10^5 thymocytes stimulated with varying amounts of purified IL 1 in the presence of 5 U or 20 U of recombinant human IL 2 (panel *B*). In this case, the line with the best fit had a correlation coefficient of $r^2 = 0.983$. Thus, when providing excess IL 2 to the cultures, i.e., IL 2 concentrations that resulted in a plateau mitogenic response (9880 + 1300 cpm in panel *A* and 1800 + 280 cpm in panel *B*), the level of proliferation was strictly dependent on the amount of IL 1 added.

Inhibition of thymocyte proliferation. Recently, highly specific goat antibodies to IL 1 were developed (17). The anti-IL 1 IgG fraction has been shown to inhibit specifically IL 1-induced proliferation but not the IL 2-dependent proliferation of an IL 2-dependent cell line (17). The anti-IL 1 IgG fraction inhibited in a dose-dependent manner IL 1-induced thymocyte proliferation in the presence of excess amounts of IL 2 (Table I). The proliferative response resulting from the concerted action of IL 1 and IL 2 was inhibited at least as well as the costimulation by IL 1 and Con A.

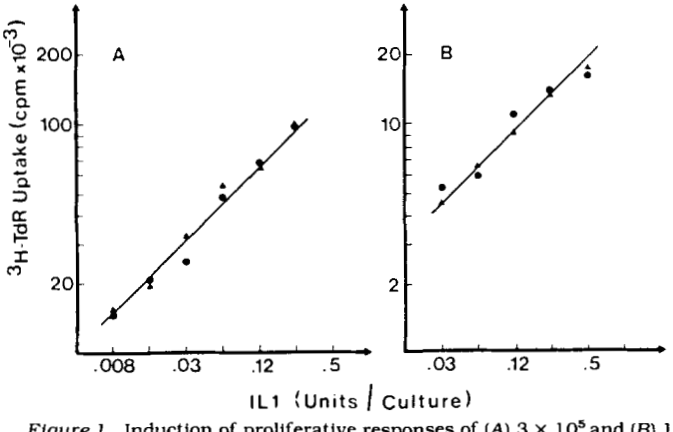

Figure 1. Induction of proliferative responses of (*A*) 3×10^5 and (*B*) 1×10^5 C3H/HeJ thymocytes with twofold serial dilutions of purified IL 1. In *A*, 10 U of partially purified murine IL 2 (▲) or 5 U of recombinant human IL 2 (●), and in *B*, 5 U (▲) or 20 U (●) of recombinant human IL 2 were present in the cultures. Recombinant IL 2 alone in *B* resulted in ^{3}HTdR incorporation without any dose dependency (cpm × 10^{-3} uptake by 50 U = 2.0 ± 0.1; 25 U = 1.7 ± 1.0; 12.5 U = 2.2 ± 1.0; 6 U = 1.6 ± 0.1; 3 U = 2.1 ± 0.7).

Induction of IL 2 receptor expression on thymocytes by IL 1 and IL 2. The T cell proliferative response to IL 2 was found to be initiated via the interaction of IL 2 with its receptor on activated T cells (20). Monoclonal antibodies (AMT 13) directed against the murine IL 2 receptor (19) were employed to measure the amount of IL 2 receptors on the cell surface. Table II shows that in the presence of IL 1 and IL 2, significant numbers of AMT 13 binding sites were induced. Neither IL 1 nor IL 2 by itself induced significant amounts of IL 2 receptor on thymocytes. A concentration of AMT 13 hybridoma supernatant of 50% inhibited the IL 1-induced proliferation to background levels (41,580 cpm to 3770 cpm).

Target cells for the IL 1-induced thymocyte proliferation. As reported earlier (13), the PNA$^+$ subpopulation of the thymus does not produce IL 2 in response to stimulation with IL 1 and mitogen. Thus, this relatively immature population does not seem to be the target cell population for IL 1-mediated IL 2 production. To answer the same question for the target cell of the IL 1-induced mitogenesis in the presence of IL 2, C3H/HeJ thymocytes were stained with subagglutinating doses of PNA-FITC and were separated with a cell sorter (12). Before sorting, 16% of the thymocytes were only weakly positive (mean intensity 84) and 79% were strongly fluorescent (mean intensity 525). Analysis of the sorted cells indicated that each population was greater than 95% pure. The data in Table III demonstrate that PNA$^+$ cells do not acquire IL 2 responsiveness when exposed to IL 2. The synergistic

TABLE I

Inhibition of IL 1-induced proliferation by anti-IL 1

Sample[a]	Anti-IL 1 IgG[b] (mg/ml)			
	0	0.5	0.25	0.125
IL 1 + IL 2	60.1 ± 1.5	8.9 ± 0.4 (85)	25.9 ± 1.2 (57)	39.8 ± 1.5 (33)
IL 1 + Con A	5.2 ± 1.7	1.7 ± 0.1 (67)	2.3 ± 0.0 (57)	3.6 ± 0.5 (33)

[a] Purified murine IL 1 was present at 0.5 U/ml in the C3H/HeJ thymocyte cultures (3×10^5 cells/culture) together with either recombinant human IL 2 (20 U/ml) or Con A (1 μg/ml).

[b] Results are given as cpm × 10^{-3} ± SD ^{3}HTdR uptake. The numbers in parentheses represent the percent inhibition of thymocyte proliferation.

TABLE II

Induction of IL 2 receptor expression on thymocytes

Sample[a]	AMT13 Binding[b] (cpm)
Medium	2,250 ± 120
IL 1	2,230 ± 760
IL 2	5,480 ± 410
IL 1 + IL 2	29,650 ± 2,330
Con A supernatant	145,680 ± 8,740

[a] Cultures of 2×10^6 thymocytes were supplemented with murine IL 1 (0.5 U/ml) and/or purified murine IL 2 (40 U/ml) or with 30% supernatant of C3H spleen cells (10^7/ml) incubated for 24 hr in the presence of Con A (5 μg/ml).

[b] Specific binding of monoclonal antibodies directed against the murine IL 2 receptor was measured on 4×10^5 cells/sample.

TABLE III
IL 1-induced proliferation of thymic subpopulations[a]

Sample[b]	Unsorted	PNA$^-$	PNA$^+$
IL 2	7.7 ± 0.7	16.6 ± 1.3	1.2 ± 0.1
IL 1	0.3 ± 0.0	0.9 ± 0.2	0.8 ± 0.1
IL 2 + IL 1	127.1 ± 1.9	197.8 ± 2.1	2.8 ± 0.8
IL 2 + Con A	85.8 ± 2.8	141.8 ± 7.0	2.3 ± 0.1

[a] Results are given as cpm × 10^{-3} ± SD ^{3}HTdR uptake.
[b] Cultures of 10^5 unseparated or PNA$^-$ or PNA$^+$ C3H/HeJ thymocytes were supplemented with recombinant human IL 2 (20 U/ml) and/or purified murine IL 1 (0.5 U/ml) and/or Con A (1 μg/ml).

effect of IL 1 and IL 2 seen with the unseparated thymocytes is also observed with PNA$^-$ subpopulation. IL 2 alone was also able to induce substantial proliferative responses in the PNA$^-$ population. However, this response was small compared with the synergistic activity of IL 1 and IL 2.

DISCUSSION

Although the involvement of IL 1 in IL 2 production by murine T cells is well established (6), the precise mechanism has not yet been elucidated. It is well documented that the sustained proliferation of activated T cells is a property unique to IL 2 (4, 20). Thus, one mode of action of IL 1 seems to be the IL 1-induced production and utilization of IL 2 by activated T cells. The data presented in this report show, however, that IL 1 may have an additional role in T cell activation. Induction of IL 2 by IL 1 (an action previously demonstrated with mature PNA$^-$ thymocytes (13)) cannot explain the synergism between IL 1 and IL 2. All thymocyte cultures contained excess amounts of purified IL 2 in order to avoid the enhancing effect of additional IL 2 produced upon the IL 1 stimulus. The utilization of purified IL 2 preparations from different sources and the use of C3H/HeJ endotoxin-low responder mice make it unlikely that other contaminating factors or lipopolysaccharide are responsible for this mitogenic effect of IL 1.

The proliferative response of thymocytes was strictly dependent on the IL 1 concentration in the cultures (Fig. 1) and was independent of the IL 2 source or concentration. The possible effect of contaminating mitogen was further ruled out by the dose-dependent inhibition of the proliferative response by specific anti-IL 1 IgG (Table I).

Induction of IL 2 responsiveness by IL 1 seems to be mediated by a concerted action of IL 1 and IL 2 on the induction of IL 2 receptors. It is not clear whether this is a direct synergistic effect of IL 1 and IL 2 or an effect mediated by another factor.

The PNA$^-$ medullary type of thymocytes appears to be the target population of IL 1-mediated IL 2 production (13). The same thymic subpopulation was found to be responsive to the IL 1/IL 2 synergism described in this report (Table III). The proliferative responses of PNA$^-$ thymocytes correlated qualitatively with those of unseparated thymocytes but were significantly higher. Cultures containing PNA$^+$ thymocytes were unable to use IL 2 upon a mitogenic stimulus. This is in contrast to earlier reports by Conlon et al. (13) but can be explained by the higher purity of the PNA$^+$ cells recovered from cell sorting as opposed to precipitation of agglutinated cells (12). Thus, the data reported here clearly suggest that in addition to promoting IL 2 production by activated T cells, IL 1 may play an additional augmenting role in T cell activation. Because IL 1 itself does not induce IL 2 receptor expression on the cell surface, the mechanism of this IL 1 mitogenic effect seems to be more complex. Under our experimental conditions, IL 2 could be required to expand the few PNA$^-$ thymocytes that express IL 2 receptors after induction by IL 1. However, pulse kinetics with PNA$^-$ thymocytes revealed that short exposure to IL 1 (up to 16 hr) did not induce measurable IL 2 receptors, and after longer exposure to IL 1 alone, cell viability was drastically decreased. How exactly IL 1 cooperates with IL 2 to induce IL 2 receptor expression within a PNA$^-$ thymocyte population will be the subject of further studies.

Acknowledgments. We thank Mr. Klaus Hexel for his assistance in cell sorting and Cetus Corporation, Emeryville, CA, for providing the recombinant human interleukin 2 preparation. We also thank Mrs. Jutta Rami for her accurate typing of this manuscript.

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