

PRODUCTION OF A CYTOKINE WITH
INTERLEUKIN 3-LIKE PROPERTIES AND CYTOKINE-
DEPENDENT PROLIFERATION IN HUMAN AUTOLOGOUS
MIXED LYMPHOCYTE REACTION

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The proliferative response elicited from cultured human T lymphocytes by the presence of autologous non-T lymphocytes has been described as being an autologous mixed lymphocyte reaction (AMLR)¹ by many laboratories (1, 2). This reactivity and the ability of murine, rat, guinea pig, or rabbit T lymphocytes to proliferate in response to syngeneic non-T stimulator cells in vitro (the syngeneic mixed lymphocyte reaction [SMLR]) are thought to represent a self-recognitive mechanism that might be important in regulating the cellular interactions involved in the generation of normal immune responses (2, 3). B lymphocytes, macrophages, or dendritic cells may stimulate this T lymphocyte response (2, 4). The responding cells belong to the helper/inducer class of T cells (5-7). The reaction is dependent on the expression of I region-associated (Ia) antigens, inasmuch as the addition of haplotype-specific anti-Ia sera to the cultures inhibits the reactions (8, 9).

The nature of the major antigenic stimulus in the AMLR has, however, been disputed. It has been proposed that previous exposure of responding T cells to foreign antigens such as sheep red blood cells (SRBC) and fetal calf serum (FCS), which are generally used during cell purification or culture, could be responsible for the observed proliferative response (10, 11). On the other hand, several data derived from human, murine, or guinea pig systems argue against this possibility and indicate that the AMLR or SMLR is a true T cell proliferative response, which is directed to Ia antigens on the stimulator cells (12-14).

Nevertheless, there is another argument against the specific nature of the AMLR or SMLR, and this is the disagreement concerning what soluble mediators are involved in the AMLR. Several investigators (15-18) have demonstrated the production of T cell growth factor (or interleukin 2, [IL-2]) in the human AMLR or mouse SMLR. However, the relationship between the IL-2, if detected, in the murine SMLR and the proliferative response in the reaction is unknown (17,

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¹ *Abbreviations used in this paper:* AHS, autologous human serum; allo-MLR, allogeneic mixed lymphocyte reaction; AMLR, autologous mixed lymphocyte reaction; CSF, colony-stimulating factor; FPLC, fast protein liquid chromatography; MMC, mitomycin C; NY, nylon wool; RPMI/AHS, RPMI 1640 medium containing autologous human serum; SMLR, syngeneic mixed lymphocyte reaction.

18). In addition, others (19, 20) have demonstrated production of some helper factors distinct from IL-2 that facilitate the development of cytotoxic cells, but have failed to demonstrate the production of a significant amount of IL-2 in the murine SMLR.

We (21) have recently demonstrated that the murine SMLR that uses responder T cells and stimulator non-T cells, which were prepared and cultured in the absence of any xenogeneic protein stimulation, induces a significant proliferative response and produces a hematopoietic growth regulator, interleukin 3 (IL-3), but not IL-2. These studies have also demonstrated that the murine SMLR may be primarily a cell interaction, in which the non-T cells stimulate helper T cells to produce IL-3, which in turn induces proliferation of IL-3-responding cells, but not mature T cells, that appear to have a phenotype of relatively early precursors in lymphocyte differentiation.

The purpose of this study was to carefully analyze the human AMLR, particularly examining whether the proliferative response in the AMLR system can be induced by using cell separation methods and culture in the absence of xenantigens, and what kinds of soluble factors regulating the reaction are produced. The results provide evidence that AMLR in the absence of any added foreign antigens generates no IL-2 production, but does generate production of a cytokine with murine IL-3-like properties, which mediates cell proliferation in the reaction.

Materials and Methods

Preparation of Responder and Stimulator Cells in AMLR. Mononuclear cells were isolated from the venous blood of healthy normal volunteers by Ficoll-Isopaque gradient centrifugation, as described (22), except that autologous human serum (AHS), was used instead of FCS in making the Ficoll-Isopaque gradient. The separated mononuclear cells were washed three times with RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) and were suspended with culture medium containing 20% AHS (RPMI/AHS).

To separate nonadherent responder T cells and adherent stimulator cells, a 20-ml glass syringe was packed with 5 g nylon wool (NY) (Leuko-Pak leukocyte filter, Travenol Laboratories, IL), drained with RPMI/AHS, and equilibrated in a 37°C incubator for 60 min. To this column, $1-3 \times 10^8$ mononuclear cells in 2-4 ml of RPMI/AHS medium were added drop by drop. The column was then washed with an additional 1 ml of warm medium. The column was then incubated at 37°C for 60 min in a 5% CO₂/95% air humidified atmosphere. The nonadherent cells were eluted with 40-60 ml of warm RPMI/AHS media at a flow rate of one drop per second, and used as responder cells (23). Responder cell fractions thus obtained contained >82% T cells as determined by the reaction with T3 monoclonal antibody and a FACS analyzer (Becton, Dickinson & Co., Sunnyvale, CA), and <0.4% B cells as detected by surface immunoglobulins.

To obtain stimulator cells, NY-adherent cells were recovered by transferring the NY wool to a Petri dish containing AHS-free RPMI 1640 medium and teasing it apart with forceps. The cells collected in the centrifuge tube were then spun for 10 min at 200 g and suspended in the RPMI/AHS medium. The cells were treated with mitomycin C (MMC) at 25 µg/10⁷ cells for 60 min at 37°C and then used as stimulator cells. These non-T cell fractions contained ~60% surface Ig⁺ B cells, 15-25% monocytes, and <8% T lymphocytes, the rest being non-T, non-B cells.

Cultured Conditions for AMLR. RPMI 1640 medium was supplemented with 20% heat-inactivated (56°C, 45 min) fresh AHS. In addition, culture medium contained 80 mM L-glutamine (Sigma Chemical Co., St. Louis, MO) and 5×10^{-5} M 2-mercaptoethanol. These conditions were used throughout the experiments except where otherwise stated. To obtain the culture supernatants for interleukin and interferon (IFN) assays, 2.5×10^6

responder T cells plus 2.5×10^6 MMC-treated stimulator cells were incubated in 24-well flat-bottomed culture plates (Nunc, Roskilde, Denmark) with a total volume of 2.0 ml. Cells and supernatants were recovered by centrifugation at 200 g for 10 min. The supernatants were passed through 0.2- μ m filters (Millipore Corp., Bedford, MA), and then tested for IL-2, IL-3, and IFN activities.

For determination of DNA replication, 200 μ l of the cell suspensions were cultured in 96-well round-bottomed microtiter plates (Falcon Labware, Oxnard, CA) at 37°C. 18 h before harvest, 0.5 μ Ci of tritiated thymidine (specific activity, 5 Ci/mmol; the Radiochemical Centre, Amersham, England) was added to each well, and the amount of radioactive uptake was measured. Data are expressed as the mean counts per minute and standard deviation of triplicate cultures.

Allogeneic MLR Assays. Allogeneic MLR was tested by using NY-nonadherent responder cells from a donor and MMC-treated NY-adherent stimulator cells from another allogeneic donor, both of which were prepared as described above. Fresh autologous serum from a responder donor, but not FCS was used for the cell separation medium. The cultured cells and their supernatants were also obtained as described in AMLR.

Assays for IL-3 Activity. The assay for IL-3-like activity was performed as previously described with the murine IL-3-dependent cell line, 32Dcl (24–26). Briefly 10^4 32Dcl cells were cultured in a final volume of 200 μ l in the presence of several concentrations of material being tested for IL-3 activity. After 21–24 h of culture at 37°C, each culture was pulsed overnight with 0.5 μ Ci of [3 H]TdR, harvested onto glass fiber filters, and counted for radioactive thymidine uptake. The results are expressed as the mean and standard deviation (SD) of specific [3 H]TdR incorporation (cpm) in triplicate cultures, as described (26).

Mouse IL-3 was purified from WEHI-3 culture supernatants by DEAE cellulose chromatography followed by chromatography on Sephacryl S-200 column and a reverse-phase high-performance liquid chromatographic system, as previously described (26). This partially purified IL-3 had a specific activity of 1.2 ng/U measured by proliferation of 32Dcl cells, and was used as a standard preparation for IL-3 activity.

IL-2 and IL-2 Assays. Human recombinant IL-2 (rIL-2) with a specific activity of 1.0×10^7 U/mg (27) was supplied by Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan) and used as a standard IL-2. The IL-2-dependent NK cell line NK-7 (27, 28) used in routine assays for the detection of IL-2 activity was maintained in RPMI 1640 containing 10% FCS and 50 U/ml of rIL-2. Another IL-2-dependent T cell line, CTLL-2 (29), was used for some experiments and maintained in RPMI 1640/10% FCS plus 1% sodium pyruvate and 50 U/ml of rIL-2.

Using these cell lines, the assays of IL-2 activity were carried out as previously described (30). Results are expressed as the mean and SD of specific [3 H]TdR incorporation (cpm) in triplicate cultures, as described (30). The data are then expressed in units (U) by comparing experimental probit data with that obtained with the use of a standard rIL-2 preparation. 1 U of a standard IL-2 is the concentration necessary to stimulate 50% of the maximum [3 H]TdR uptake by NK-7 cells (30). IL-2 levels of 0.05 U/ml or greater are significant by probit analysis. Murine monoclonal antibody against human rIL-2 was supplied by Shionogi Pharmaceutical Co. Ltd. One unit of anti-rIL-2 was defined as the dose required to neutralize one unit of rIL-2 (Yoshida et al., manuscript in preparation).

IFN- γ , anti-IFN- γ Serum, and IFN Assay. Human recombinant IFN- γ with a specific activity of 1.0×10^7 U/mg and monoclonal antibody against recombinant human INF- γ (27) were kindly supplied by Shionogi Pharmaceutical Co. The 1:10,000 antiserum neutralized natural and recombinant human IFN- γ at 10 U/ml, but the antiserum, even undiluted, could not neutralize human recombinant IFN- α or IFN- β at 10 U/ml.

Antiviral activity was assayed on human WISH cells by the 50% plaque reduction method and expressed as reciprocal values of the dilutions that reduce the numbers of virus plaques by 50%, as previously described (21).

Monoclonal Reagents. Monoclonal antibodies of the OK series, i.e., OKT3 (anti-panT), OKT4 (anti-helper/inducer T), and OKT8 (anti-suppressor/cytotoxic T), were purchased from Ortho Diagnostics (Raritan, NJ). Cells reactive with these antibodies were

enumerated by an indirect immunofluorescence and FACS. A monoclonal antibody to HLA-DR antigens (OKIa1) was purchased from Ortho Diagnostics. This antibody is directed against the complex of both chains of DR antigens and it binds to B cells and macrophages but not to normal T cells.

Elimination of OKT4 or OKT8 Cell Population by Complement-mediated Lysis. Lysis of T cell populations by monoclonal antibodies was done as previously described (31) with a minor modification. Briefly, test cells were treated with 0.1 ml of OKT4 or OKT8 antibody (100 μ g of protein/ml) and 1.0 ml of 1:10-diluted rabbit C (Lo-Tox-H rabbit complement, Cederlane Laboratories, Ltd., Hornby, Ontario, Canada) in RPMI/AHS for 45 min at 37°C. The surviving cells were then washed three times, counted, and tested for their response to AMLR stimulators. When T cell-enriched responder fractions were treated with anti-T4 and C, residual cells consisted of >90% T3⁺ and T8⁺ cells, with <2% T4⁺ cells. Treatment of cells with anti-T8 and C yielded preparations consisting of >90% T3⁺ and T4⁺ cells and <3% T8⁺ cells.

General Biochemical Procedures. Protein determinations throughout the purification employed were based on the OD at 280 nm. The AMLR culture fluids were condensed by ultracentrifugation using the Diaflow membrane YM-5 (Amicon Corp., Lexington, MA). Gel filtration analysis of these condensed AMLR culture fluids was performed by using a Superose 12 column of the fast protein liquid chromatography (FPLC) system (Pharmacia Fine Chemicals, Uppsala, Sweden). DEAE cellulose chromatography was done by using a MonoQ column of the FPLC system (Pharmacia Fine Chemicals). For both chromatographies, the condensed AMLR culture fluids were dialyzed extensively against equilibration buffer, and applied to the column. The Superose 12 column was equilibrated and developed with 10 mM phosphate-buffered saline (PBS, pH, 7.2), and 0.5 ml of effluents at each fraction were collected and then assayed for cell growth-stimulating activities. The M_r of the activities in this gel filtration were calibrated by using the standard markers, ferritin (M_r 440,000), aldolase (M_r 158,000), bovine serum albumin (M_r 67,000), and ribonuclease A (M_r 13,700). The MonoQ column was eluted with a linear gradient of 0–0.5 M NaCl. Fractions of 0.5 ml were collected and the biological activities of each were determined.

Bone Marrow Cultures. Bone marrow was obtained from healthy volunteers by aspiration from the iliac crest. Light-density cells were separated by Ficoll-Isopaque density gradient centrifugation. Cells were washed with the buffer and suspended at 5×10^6 cells/ml in RPMI 1640 medium containing 10% FCS. To 100 μ l of cell suspensions seeded in wells of a 96-well microtiter plate (Falcon Labware) was added 100 μ l of test materials at twofold dilutions and then incubated at 37°C for 72 h. Cultures were harvested onto glass fiber filter papers and counted by the liquid scintillation technique for thymidine incorporation as described.

Results

DNA Replication in AMLR in the Absence of Foreign Proteins. Table I shows the results of experiments, in which NY-nonadherent T lymphocytes from six healthy volunteers were cultured with MMC-treated autologous NY-adherent stimulator cells in a culture medium containing AHS for 5 d and then examined for DNA replication. With all the cases, the responder cells cocultured with the stimulator cells generated significant DNA replication. The cultures of responder cells alone produced no definite DNA replication in the absence of stimulator cells, nor was there any significant DNA replication in the cultures of stimulator cells alone. These results indicate that the cultivation of responder T cells with adherent non-T stimulator cells in the absence of any xenogeneic protein antigens produces a significant AMLR. When graded numbers of nonadherent responder cells (2×10^6 to 1.25×10^5) were cultured with 5×10^5 of MMC-treated stimulator

TABLE I
 AMLR in Healthy Individuals

Donors	^3H TdR incorporation (cpm)		
	Responder + stimulator	Responder	Stimulator
RS	49,653 \pm 2,264	672 \pm 307	265 \pm 54
MI	50,012 \pm 7,356	1,448 \pm 639	198 \pm 17
YT	34,792 \pm 4,081	747 \pm 319	318 \pm 199
KH	43,281 \pm 4,930	519 \pm 266	215 \pm 88
TT	30,986 \pm 2,736	398 \pm 101	164 \pm 47
RS	40,861 \pm 708	601 \pm 52	291 \pm 86

Responder cells (5×10^5) from each individual were incubated with MMC-treated stimulator cells (5×10^5) in a 200- μl culture medium containing AHS for 5 d and examined for DNA replication. Responder cells or stimulated cells alone were also cultured for 5 d.

TABLE II
 Effect of Anti-DR Monoclonal Antibody in Induction of AMLR and Allo-MLR

Dilutions of anti-DR added	AMLR ^3H TdR incorporation*	Inhibition ‡	Allo-MLR ^3H TdR incorporation*	Inhibition ‡
	cpm	%	cpm	%
Medium	46,097 \pm 8,527	—	75,664 \pm 4,363	—
1:10	8,051 \pm 2,901	82.5	9,633 \pm 1,749	87.2
1:100	29,756 \pm 3,046	35.4	37,627 \pm 10,209	50.2
1:1,000	43,666 \pm 10,551	5.2	65,263 \pm 7,209	13.8

* Responder nonadherent T cells (5×10^5) from a donor were incubated with the adherent stimulator cells (5×10^5) from an autologous or allogeneic donor in the presence of anti-DR antibody at the final dilutions indicated for 5 d, and then examined for their ^3H TdR incorporations. Each value is the mean \pm standard deviation of three determinations.

‡ Percent inhibition of medium controls.

cells, the maximum replication was obtained with 5×10^5 responder cells (data not shown).

Inhibition of the AMLR Response by Monoclonal Anti-DR Antibody. It has been reported that in the SMLR or AMLR, as well as the allogeneic (allo) MLR, the class II major histocompatibility complex (MHC) gene products (Ia or HLA-DR antigens) are critically important in stimulating this response (8, 9, 13, 14). It has also been shown that the responder cells in human AMLR are T4⁺, T8⁻ helper/inducer type of T cells (32, 33). We therefore examined whether the AMLR induced in our systems also involves an interaction between the HLA-DR⁺ stimulator cells and responder/helper T cells, by using monoclonal antibodies. As shown in Table II, when the responder T cells were incubated with autologous or allogeneic stimulator cells in the presence of anti-DR monoclonal antibody, the proliferative response in AMLR, as well as in allo-MLR, was significantly inhibited by the antibody in a dose-related fashion.

Subsequently, nonadherent T cells were treated with complement and T4 or T8 monoclonal antibody, and examined for DNA replication in response to autologous stimulator adherent cells (Table III). Nonadherent T cells that had been depleted of T4 but not of T8 cells produced an extremely reduced DNA

TABLE III
*Abrogation of Proliferative Response of Responder Cells in AMLR
 after Treatment with Complement and Anti-T4 but Not Anti-T8
 Monoclonal Antibody*

Treatment	[³ H]TdR incorporation*	Inhibition [†]
	<i>cpm</i>	<i>%</i>
C	42,230 ± 2,793	—
Anti-T4 + C	7,739 ± 1,155	81.7
Anti-T8 + C	47,931 ± 4,458	0

The C-treated, or C plus anti-OKT4- or OKT8-treated nonadherent T cells were reconstituted to 10⁶ lymphocytes/ml, and used as responder cells in AMLR. [³H]TdR incorporation was measured on day 5 of culture.

* Each value is the mean ± standard deviation of three cultures.

[†] Percent inhibition of medium control.

replication, indicating that the T4⁺T8⁻ helper/inducer T cells are involved in DNA replication in AMLR as previously described (31, 32).

Production of a Lymphokine with IL-3-like Properties but Not IL-2 or IFN in AMLR. We next examined, in addition to DNA replications, production of IL-2 and IFN, and the murine IL-3-like activity assayed by 32Dcl cell line, in the AMLR supernatants of the coculture of responder T cells with stimulator non-T cells in a culture medium containing AHS. These results were compared with those for allo-MLR, in which the responder T cells from the same donor were incubated with the stimulator cells isolated from another donor. The results of a representative experiment shown in Fig. 1A indicate that DNA replication in AMLR was demonstrable on day 3 after incubation, and reached a peak on day 5, decreasing thereafter. Regardless of this active proliferative response, no minimal levels of IL-2 (0.05 U/ml) and IFN (2 U/ml) could be detected during the entire culture periods, but the activity stimulating the growth of a murine IL-3-dependent cell line 32Dcl was found. The activity to a minimal level was detected as early as 1 d after incubation in AMLR, increased with time, and reached the maximum (18 × 10³ cpm) on day 5. On the other hand, in alloMLR (Fig. 1B), IL-2 at ~10 U/ml and IFN at 80 U/ml could be induced. Production of the activity that stimulated 32Dcl cells with a maximum of 5 × 10³ cpm on day 5 was also induced.

To confirm that there was no production of IL-2 in the AMLR, the AMLR supernatants on day 3 were condensed into a 0.1 vol by a Centricon membrane and tested for IL-2 activity in their serial dilutions using CTLL-2 and NK-7 cell lines, and for activity stimulating the murine IL-3-dependent cell line 32Dcl (Fig. 2). The human rIL-2 served as controls (Fig. 2). The condensed culture fluids were found to contain an increased amount of murine IL-3-like activity stimulating the target, but no IL-2 assessed by either CTLL-2 or NK-7, both of which were responsible for rIL-2 stimulation. With the culture fluids harvested on day 1, 2, or 4, no IL-2 activity was detected. Also, no IFN activity was found in any condensed AMLR culture fluids (data not shown).

No Involvement of IL-2 and IFN-γ in DNA Replication in the AMLR. We further examined whether IL-2 and IFN, which might be produced in the AMLR to

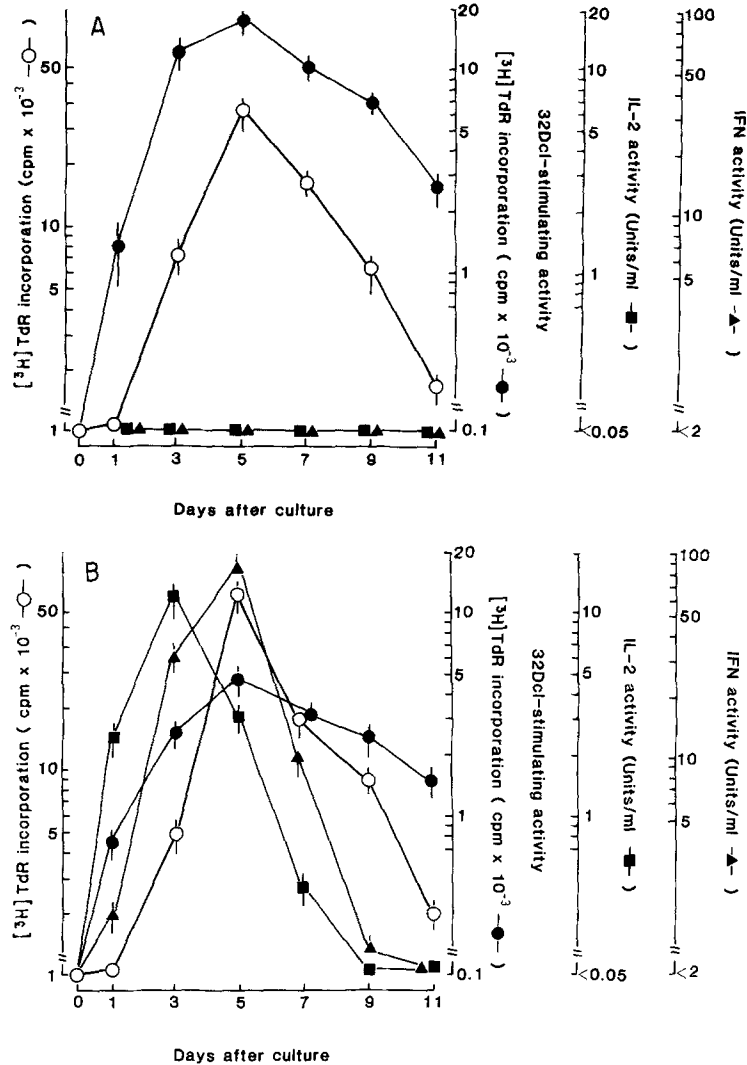


FIGURE 1. A time course of DNA replication and lymphokine production in (A) AMLR and (B) allo-MLR. Responder NY-nonadherent T cells (2.5×10^6) from a donor were incubated with 2.5×10^6 stimulator NY-adherent cells of an (A) autologous or (B) allogeneic donor in 2.0 ml of RPMI/AHS at 37°C for 11 d. At the indicated time after incubation, activity to stimulate 32Dcl cells (\bullet), IL-2 activity by NK-7 cell growth (\blacksquare), and IFN activity (\blacktriangle) in the culture supernatants were measured. [^3H]TdR incorporation by the cultured cells (\circ) were assayed in a $200\text{-}\mu\text{l}$ culture system described in Materials and Methods. Values represent the means \pm standard deviation of triplicate cultures.

undetectable levels, are involved in the development of DNA replication in AMLR. Specific monoclonal antibodies directed against human rIL-2 or human rIFN- γ were added to the culture, and then tested for their effect on the proliferative response. The cultures of alloMLR in the presence of anti-IL-2 or anti-IFN- γ served as controls (Table IV). The results clearly showed that both 2.5–10 U/ml of anti-rIL-2 and 25–50 U/ml of anti-rIFN- γ did not affect the

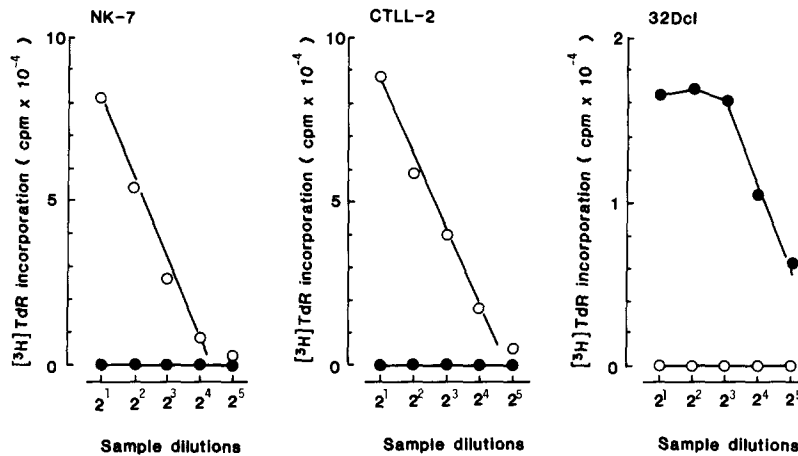


FIGURE 2. Function of AMLR supernatants in maintaining murine IL-3-dependent cell lines 32Dcl but not IL-2-dependent cell lines NK-7 or CTLL-2. 10-fold condensed AMLR supernatants (day 3) (●), or human rIL-2 (5 U/ml) (○) were titrated into the cultures and the proliferative response was measured. The values represent the means of triplicate cultures.

TABLE IV
Effect of Anti-IL-2 and Anti-IFN- γ Monoclonal Antibodies in Induction of
AMLR and Allo-MLR

Donor	Incubation with:	[³ H]TdR incorporation (cpm \pm SD)	
		AMLR	Allo-MLR
A	Medium	52,194 \pm 6,013	71,358 \pm 16,254
	Anti-IL-2 (10 U/ml)	48,618 \pm 1,625 (7)*	14,385 \pm 4,209 (80)
	(2 U/ml)	52,164 \pm 5,366 (<1)	19,068 \pm 7,586 (73)
	Anti-IFN- γ (50 U/ml)	50,393 \pm 2,369 (3)	27,348 \pm 13,609 (62)
	(25 U/ml)	50,624 \pm 2,634 (3)	39,981 \pm 12,033 (43)
B	Medium	43,754 \pm 5,322	ND
	Anti-IL-2 (10 U/ml)	42,253 \pm 7,061 (3)	ND
	(5 U/ml)	42,958 \pm 5,274 (2)	ND
	(2.5 U/ml)	49,581 \pm 1,097 (0)	ND
	Anti-IFN- γ (50 U/ml)	44,214 \pm 2,550 (0)	ND

Responder cells (5×10^5) from a donor were cultured with 5×10^5 adherent stimulator cells from an autologous (AMLR) or allogeneic (AlloMLR) donor in the 200 μ l of culture medium in the absence or presence of anti-IL-2 or anti-IFN- γ monoclonal antibodies at the titers indicated. On day 5, [³H]TdR incorporation by the cultured cells was assayed. Values represent the means \pm standard deviation of triplicate cultures.

* Numbers in parentheses represent percent inhibition of medium control.

proliferative response of AMLR, whereas they definitely diminished the proliferation of cells in the allo-MLR in a dose-related fashion. These results indicate that DNA replication in AMLR, unlike allo-MLR, did not involve either IL-2 or IFN- γ in the reaction.

IL-3-like Activity as a Growth Factor Responsible for AMLR. We further examined whether the IL-3-like activity produced in the AMLR culture fluids is a growth factor responsible for the AMLR-induced DNA replication, which was found to be independent of IL-2 and IFN. First, we harvested the AMLR culture

TABLE V
Proliferative Response of PBL to the AMLR Culture Supernatants

Mononuclear cells	AMLR supernatant added (25%)	[³ H]TdR incorporation (cpm)
Unfractionated	+	3,986 ± 646
	-	220 ± 35
NY-nonadherent	+	16,092 ± 2,730
	-	241 ± 54
NY-adherent	+	1,776 ± 734
	-	271 ± 66

The culture fluids in AMLR on day 4 were harvested, and 50 μ l of the culture fluids was added to 150 μ l of culture medium containing unfractionated, NY-nonadherent, and NY-adherent responder mononuclear cells in wells of a 96-well microplate, cultured for 60 h, labeled with [³H]-TdR, and then examined for their [³H]TdR incorporation 12 h after incubation. The values represent the means \pm standard deviation of triplicate cultures.

fluids on day 3, added them to peripheral blood mononuclear cells (unfractionated) and their adherent and nonadherent fractions, and incubated for 3 d to examine for their proliferative responses (Table V). The nonadherent fraction, and unfractionated cells to a lesser extent, but not adherent mononuclear cells, responded to the AMLR culture fluids with proliferation.

Next, the AMLR culture fluids on day 5 were condensed and subjected to chromatography on FPLC-Superose 12. The effluents were tested for activity supporting the proliferation of 32Dcl and activity inducing the proliferation of nonadherent responder cells (Fig. 3). The results show that both activities were eluted from the column as a single peak at fraction 31 (corresponding to an M_r of 15,000–28,000). Neither IL-2 nor IFN was found in any effluents of AMLR culture fluids from the column (data not shown).

The AMLR culture fluids concentrated by an Amicon membrane were also applied to a chromatography on a MonoQ column of the DEAE-type FPLC system. The column was subsequently eluted with a linear concentration gradient of NaCl. As shown in Fig. 4, a major factor that induces proliferation of 32Dcl did not bind to the column and was eluted in the run-through fractions. The major activity inducing the replication of AMLR responder cells was eluted together with the IL-3-like activity. Both activities inducing the growth of 32Dcl, and nonadherent responder cells were also eluted as a minute fraction from the column by 0.2–0.3 M NaCl. Both fractions had no apparent effect on the proliferation of IL-2-responding CTLL-2 or NK-7 cells. The mitogenic tests for murine thymocytes revealed that the factors had no IL-1-like mitogenic activity (34 and data not shown).

Furthermore, we examined whether the IL-3-like factor acted to support replication of the growing cells in the AMLR. The cells in AMLR on day 5 were rigorously washed with the buffer, incubated further for 2 d in the FPLC-MonoQ column-purified factor, and then examined for the factor-dependent growth of test cells (Table VI). The results clearly showed that the cells during

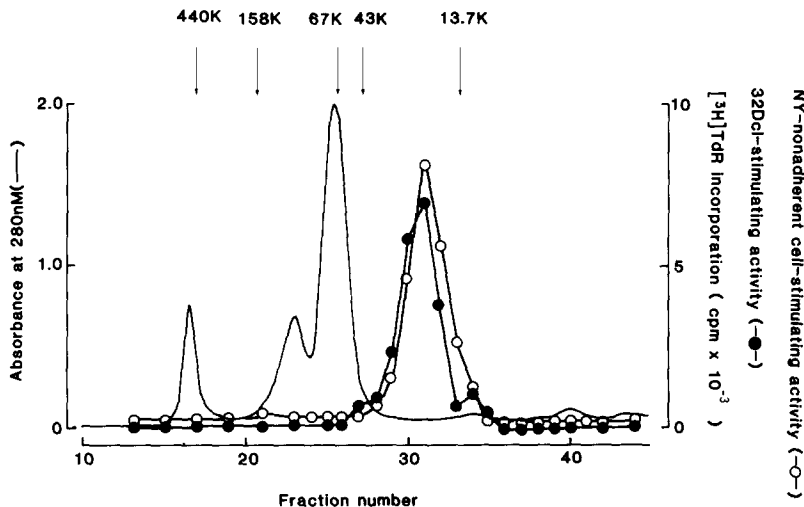


FIGURE 3. Detection of activity-stimulating 32Dcl cells or nonadherent responder mononuclear cells in AMLR in the effluents from an FPLC-Superose 12 column of AMLR culture fluids. 0.5 ml of 10-fold concentrated AMLR culture supernatants by a Centricon membrane was subjected to Superose 12 chromatography and developed with 0.01 M phosphate buffer (pH 7.2). The effluents were dialyzed against RPMI medium and then tested for IL-3-like activity by 32Dcl cell growth (●) and activity stimulating nonadherent responder cells in AMLR (○). K, standard M_r markers in thousands. The values represent the mean \pm standard deviation in triplicate assays.

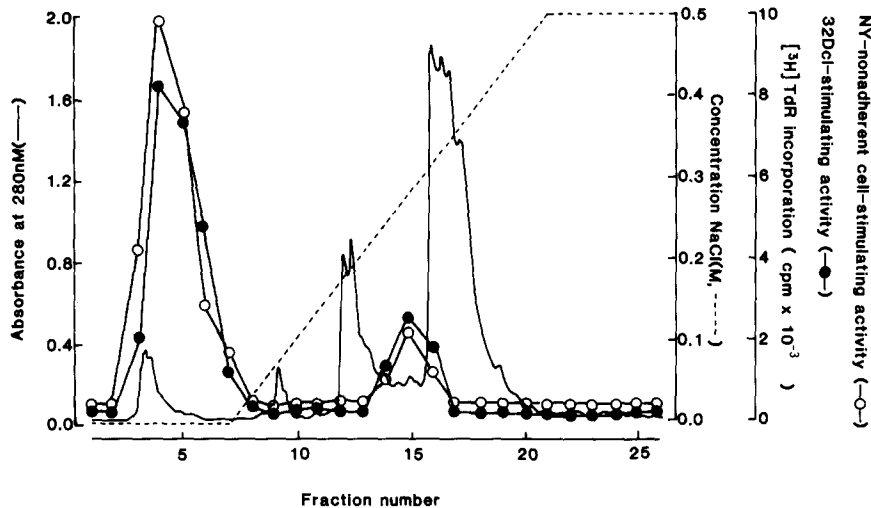


FIGURE 4. Detection of IL-3-like activity and activity-stimulating nonadherent responder mononuclear cells in the effluents from FPLC-MonoQ column of AMLR culture fluids. 0.5 ml of 10-fold concentrated AMLR culture supernatants (on day 5) were subjected to chromatography on the FPLC-MonoQ column and developed with a 0.01–0.5 M NaCl gradient in 0.01 M sodium phosphate buffer (pH 7.2). The effluents, after dialyzation against RPMI 1640 medium, were tested for stimulation of 32Dcl cell growth (●) and activity-stimulating nonadherent responder cells in AMLR (○). Values are the mean counts per minute in triplicate assays.

TABLE VI
Supporting Effect of the Factor on the Growth of Responding Cells during AMLR Cultures

Concentrations of AMLR-IL-3 added (%)	[³ H]TdR incorporation (cpm ± SD)
25	29,754 ± 756
12.5	25,868 ± 1,935
6.25	22,015 ± 1,638
3.1	18,740 ± 1,638
1.6	14,419 ± 1,207
—	9,759 ± 826

The cultured cells on day 5 of AMLR were harvested and washed three times with the buffer. The cells harvested (5×10^5) were cultured further for 2 d in fresh RPMI/AHS in the absence or presence of the factor partially purified by FPLC MonoQ column at the indicated concentrations and then examined for [³H]TdR incorporation.

AMLR culture continued to extensively grow in the medium containing the factor in a dose-dependent fashion, although they also replicated to a much lesser extent in the fresh medium without addition of the factor.

Growth-stimulating Effect of the Factor on Bone Marrow Cells. Finally, we examined whether this IL-3-like factor had any effect on stimulation of the bone marrow cell, as did the murine IL-3 (35). The bone marrow cells isolated from a healthy donor were incubated with a medium containing factor, partially purified by MonoQ column chromatography, in a serial dilution for 60 h, and then examined for their proliferative responses (Fig. 5). The results clearly showed that the factor, in a dose-related fashion, stimulated bone marrow cell proliferation. It was also shown that the proliferative response of bone marrow cells to the factor was much higher than that of peripheral blood lymphocytes, as was the response of murine bone marrow cells to the murine IL-3 (35). The experiments were repeated with bone marrow cells from five other donors with similar results.

Discussion

In the present study, we initially focused attention on the protection of AMLR from the sensitization of test cells by xenogeneic antigen stimulation. The problem of FCS as mitogen and antigen has been well recognized for a long time. FCS induces blastogenic and cytotoxic responses in unseparated human peripheral blood lymphocytes (PBL) (36). SRBC, which have generally been used for separation of T and B cells, can also stimulate an antigen-specific plaque-forming cell (PFC) response in unseparated PBL in the absence of any nonspecific stimulating agent (37). Therefore, in our experiments we used the technique of passage through NY column to separate responder T cells and stimulator non-T cells. Autologous human serum, but not FCS or allogeneic human serum, was always used for the cell separation and culture. By using these techniques, we have shown that NY-nonadherent responder cells generated a significant DNA replication in response to NY-adherent stimulator cells, without any background proliferative response in the absence of stimulator cells.

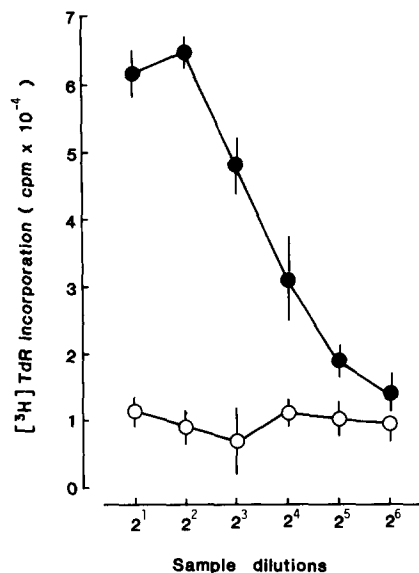


FIGURE 5. Proliferative response of bone marrow cells to the factor. The serial dilutions of active fraction (100 μ l) from the MonoQ column of AMLR culture fluids (●) or 100 μ l of RPMI/AHS medium (○) were added to 100 μ l of 2.5×10^6 /ml bone marrow cells in wells of a 96-well microtiter plate and incubated for 60 h. The cells were then examined for [3 H]TdR incorporation (12-h-incubation). Values are the mean \pm standard deviation in triplicate cultures.

Earlier studies by Huber et al. (10) and Kagan and Choi (11) reported that, in the absence of stimulation by SRBC or FCS, the human AMLR did not take place, according to the criteria of T cell proliferation. The response could be observed only in cultures that had either been supplemented with FCS or contained lymphocytes that had been previously exposed to FCS or SRBC. A more recent study by Naides et al. (38) has, however, shown that nonadherent T cell populations purified by nylon filtration or plastic adherence produce an AMLR even in the absence of exposure to xenoantigens. Although the reasons for the discrepancies among these studies, including ours, remain unknown, it is possible that the differences in the culture conditions or cell populations used might cause these discrepancies. For example, the NY-adherent non-T cells that we used as a stimulator were the same as those used by Kagan and Choi (11), but responder cells used in the study by Naides et al. (38), as well as in our study, were NY-purified nonadherent cells, which were different from the unseparated peripheral mononuclear cells used by Kagan and Choi.

The AMLR assayed in the absence of xenoantigen stimulation in the present study, as well as allo-MLR, was shown to be dependent on the expression of DR antigens and mediated by $T4^+$, $T8^-$ helper/inducer T cells. These results are consistent with previous reports by other investigators showing that the human AMLR (7, 9, 32, 33) or murine SMLR (5, 8, 14) may represent helper/inducer T cell activation mediated through a receptor for self-Ia antigens. The AMLR in our study, however, regardless of the significant DR antigen-dependent response of helper T cells, generated no production of IL-2, in contrast to

definite levels of IL-2 production in allo-MLR. Earlier studies by Palacios and Möller (15) demonstrated the production of IL-2 in an AMLR that contained lymphocytes that had been separated by rosetting using SRBC. Exposure of lymphocytes to SRBC alone stimulates significant T cell proliferation and PFC responses in the human AMLR (11). Therefore, it is not surprising that under such conditions, IL-2 production takes place. In the murine syngeneic MLR system, Lattime et al. (17, 18) reported that T cell proliferation could be detected in cultures containing FCS but not in those supplemented with syngeneic mouse serum. However, small amounts of IL-2 were produced independent of the presence of FCS. Some other reports on human autologous (11) and murine syngeneic (19, 20) MLR have, however, failed to demonstrate the production of IL-2. Our recent work (21) has shown that the murine syngeneic MLR, as well as the human AMLR shown in the present study, generated no IL-2 production, regardless of a significant DNA replication in the absence of any xenogeneic protein stimulation.

We showed further that DNA replication induced in the human AMLR was not inhibited by addition of a specific antiserum against human rIL-2, which could suppress DNA replication in the allo-MLR. Specific IFN- γ antiserum, which was shown to suppress the allo-MLR, as described in the murine systems (39), also did not affect the human AMLR. These results may also support the results showing no production of IL-2 and IFN- γ in the AMLR.

In the present study, we have found that a cytokine, which can stimulate the murine IL-3-dependent cell line 32Dcl, is produced in the AMLR soon after culture and before the onset of proliferative response. The major activity in the AMLR culture fluids had an M_r of 15,000–28,000 in gel filtration, which was similar to an M_r of human IL-2 (40), but it had no activity to stimulate IL-2-dependent cell lines NK-7 or CTLL-2. The major activity did not bind to FPLC-MonoQ column (DEAE type), to which IL-2 bound (40), and was recovered in the run-through fractions, as was the murine IL-3 (26, 35). It was also found that the AMLR culture fluids contained a soluble factor that could stimulate proliferation of the fresh nonadherent mononuclear cells. The factor, when applied to the Superose 12 and FPLC-MonoQ columns was eluted from both columns together with the IL-3-like activity. These results strongly suggest that the factor may be a major activity responsible for the cell proliferation in the AMLR.

Murine IL-3 is a multilineage hematopoietic growth regulator that initiates the proliferation and differentiation of multipotential stem cells, leading to the production of all the major blood cell types (35, 41–43). It is produced by mitogen- or antigen-activated T lymphocytes and by a number of continuous cell lines (35). Purified murine IL-3 exhibited a growth-supporting effect on IL-3-dependent myelomonocytic, lymphoid, or mast cell lines but not on any IL-2-dependent cell lines. IL-3 derived from the WEHI-3 cell cultures has been purified to homogeneity by a combined method using different kinds of chromatography (43). The protein is glycosylated and the M_r of the major glycosylated species was estimated to be 28,000 (43). Recently, cDNA clones specifying the IL-3 derived from the WEHI-3 cells have been isolated, and a polypeptide of 166 amino acids, including a putative signal peptide, has been deduced from the

cDNA (44). At present, molecules equivalent to murine IL-3 have not been isolated from the human lymphocyte cultures. The factor found in the human AMLR culture fluids in this study had a murine IL-3-like property in the activity supporting the growth of murine IL-3-dependent cell line, 32Dcl, but not in that supporting the growth of IL-2-dependent cell lines NK-7 or CTLL-2. It had no IL-2 or IFN activity. Nor was there any IL-1 activity in the factor. The factor strongly stimulated freshly isolated bone marrow cells, as did the murine IL-3 (35, 41, 42). Although human and murine colony stimulating factors (CSF), as well as murine IL-3, could stimulate bone marrow cells (45, 46), 32Dcl cells responsible for murine IL-3 and the IL-3-like factor in the present study did not respond to human recombinant granulocyte/macrophage CSF (data not shown). Some physicochemical properties, such as elution patterns in DEAE chromatography, were similar to those of murine IL-3 (42, 43). These results strongly suggest that the factor found in this study may be a human molecule(s) equivalent to murine IL-3 or a molecule closely related to it. A similar activity was found in the allo-MLR culture fluids (Fig. 1) and other antigen-specific lymphocyte cultures (data not shown). Further purification and the physicochemical properties of the factors are being examined.

In our recent experiments (21), it has been shown that in the murine SMLR, Thy-1⁺, Lyt-1⁺, 2⁻ helper/inducer T cells produce IL-3 in response to NY-adherent stimulator cells, and that the IL-3 induces proliferation of Thy-1⁻, Lyt-1⁻, 2⁻, sIg⁻, asialo GM₁-negative cells, but not of IL-2-responsive Thy-1⁺ or asialo GM₁-positive cells. These results suggest that the murine SMLR may be primarily a proliferative reaction to the IL-3 of early precursors in lymphocyte or hematopoietic cell differentiation. Although further experiments are underway that will determine the responder cells to the factor and proliferating cells in the human AMLR, our unpublished observations indicate that although the producing cells of IL-3 in response to the IL-3-like factor are T3⁺, T4⁺, T8⁻, T11⁺ helper cells, the responding cells to the factor appear to be cells with low density but lacking a typical phenotype of T cells or NK cells (Suzuki et al., manuscript in preparation). Therefore, we propose that the AMLR (SMLR) in human and murine systems may be a phenomenon, in which self-Ia antigens on the stimulator cells stimulate helper/inducer T cells to produce IL-3 or IL-3-related molecules but not IL-2, which in turn induce proliferation of the responding cells to the factor. These responding cells are not IL-2-responsive mature T cells or NK cells.

It remains unknown whether the AMLR and the selective production of IL-3 or IL-3-like molecules have a physiologic significance in regulation of normal immune responses *in vivo*. As to a possible role in lymphocyte differentiation, however, IL-3 has been shown to stimulate early events in the differentiation of T cells such as Thy-1 expression on the cell (35) or to augment the primary cytolytic T lymphocyte response to allogeneic tumor cells (47). It is presumed, therefore, that the AMLR may play a role at least in T and B lymphocyte differentiation in the immune responses, acting through production of IL-3. IL-3 has also been shown to stimulate premature B cells to produce Ig (48).

It has also been reported by several investigators that the human or murine AMLR produces soluble factors (19, 20, 49-51) that were shown to play a helper

or suppressor role in regulation of certain immune responses. Some structural homologies among certain of these factors and the IL-3-like factor found in the present study might be anticipated, although further elucidation will require additional biological and biochemical analysis.

Summary

The autologous mixed lymphocyte reaction (AMLR) was assayed in a medium containing fresh autologous serum, by using nylon-adherent stimulator cells and nonadherent responder T cells, which were prepared from human peripheral blood mononuclear cells in the absence of fetal calf serum (FCS) to avoid any sensitization to xenogeneic protein antigens. DNA replication without a background proliferative response was induced by stimulator cells in the responder cells. The addition of monoclonal anti-HLA-DR antibody to the culture or treatment of the responder cells with complement plus anti-T4 but not anti-T8 monoclonal antibody suppressed the AMLR, suggesting that this specific AMLR involves an interaction between HLA-DR antigens and helper/inducer T cells. Regardless of this specific DNA replication, the AMLR generated no production of interleukin 2 (IL-2) and interferon γ (IFN- γ), both of which could be found in the allogeneic (allo) MLR. In addition, DNA replication in the AMLR was not inhibited by the addition of specific antisera for IL-2 and IFN- γ , both of which significantly inhibited the DNA replication in allo-MLR.

The AMLR was accompanied by production of a soluble factor, which could stimulate the proliferation of murine interleukin 3 (IL-3)-dependent cell line 32Dcl but not the proliferation of IL-2-dependent cell lines. This factor was also found to be responsible for proliferation of responder nonadherent cells in the AMLR. It strongly stimulated bone marrow cells, as did the murine IL-3. The factor had an M_r range, as determined by gel filtration, of 15,000–28,000, but it did not bind to fast protein liquid chromatography (FPLC)-MonoQ column. Thus, the factor is distinguishable from IL-2 in physicochemical or biological properties, but similar to murine IL-3. These results suggest that the human AMLR may be primarily a phenomenon in which non-T cells mediated by the HLA-DR antigens on the cell stimulate helper/inducer T cells to produce a lymphokine with IL-3-like properties, but no IL-2, which in turn stimulates the factor-dependent cells to proliferate.

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References

1. Opeltz, G., M. Kiuchi, M. Takesugi, and P. I. Terasaki. 1975. Autologous stimulation of human lymphocyte subpopulations. *J. Exp. Med.* 142:1327.
2. Weksler, M. E., C. E. Moody, Jr., and R. W. Kozak. 1981. The autologous mixed lymphocyte reaction. *Adv. Immunol.* 31:271.
3. Weksler, M. E., and R. Kozak. 1977. Lymphocyte transformation by autologous cells. V. Generation of immunologic memory and specificity during the autologous mixed lymphocyte reaction. *J. Exp. Med.* 146:1833.

4. Nussenzweig, M. C., and R. M. Steinman. 1980. Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196.
5. Pastermak, R. D., M. H. Bocchieri, and J. B. Smith. 1980. Surface phenotype of responder cells in syngeneic mixed lymphocyte reaction in mice. *Cell. Immunol.* 49:384.
6. Glimcher, L. G., A. D. Steinberg, S. B. House, and I. Green. 1980. The autologous mixed lymphocyte reaction in strains of mice with autoimmune diseases. *J. Immunol.* 125:1832.
7. Kozak, R. W., C. E. Moody, L. Staiano-Coico, and M. E. Weksler. 1982. Lymphocyte transformation induced by autologous cells. XII. Quantitative and qualitative differences between autologous and allogeneic reactive T lymphocytes. *J. Immunol.* 128:1723.
8. Yamashita, U., and E. M. Shevach. 1980. The syngeneic mixed leukocyte reaction: the genetic requirements for the recognition of self resemble the requirements for the recognition of antigen in association with self. *J. Immunol.* 124:1773.
9. Hausmann, P. B., and J. D. Stobo. 1979. Specificity and function of a human autologous reactive T cell. *J. Exp. Med.* 149:1537.
10. Huber, C., M. Merckenslager, C. Gattringer, I. Royston, U. Fink, and H. Braunsteiner. 1982. Human autologous mixed lymphocyte reactivity is primarily specific for xenoprotein determinants adsorbed to antigen-presenting cells during rosette formation with sheep erythrocytes. *J. Exp. Med.* 155:1222.
11. Kagan, J., and Y. S. Choi. 1983. Failure of the human autologous mixed lymphocyte reaction in the absence of foreign antigens. *Eur. J. Immunol.* 13:1031.
12. Löffön, A., J. Alcocer-Varela, and D. Alarcin-Segovia. 1983. The autologous mixed lymphocyte reaction is not primarily due to xenoantigenic stimulation. *Clin. Immunol. Immunopathol.* 28:304.
13. Hausman, P. B., C. E. Moody, J. B. Innes, J. J. Gibbons, and M. E. Weksler. 1983. Studies on the syngeneic mixed lymphocyte reaction. III. Development of a monoclonal antibody with specificity for autoreactive T cells. *J. Exp. Med.* 158:1307.
14. DosReis, G. A., and E. M. Shevach. 1981. The mixed leukocyte reaction presents polyclonal activation of antigen-specific T lymphocytes with receptors for self-Ia antigens. *J. Immunol.* 127:2456.
15. Palacios, R., and G. Möller. 1981. HLA-DR antigens render resting T cells sensitive to interleukin-2 induce production of the growth factor in the autologous mixed lymphocyte reaction. *Cell. Immunol.* 63:143.
16. Takada, S., Y. Yeda, N. Suzuki, Y. Murakawa, T. Hoshino, I. Green, A. D. Steinberg, D. A. Horwitz, and T. Sakane. 1985. Abnormalities in autologous mixed lymphocyte reaction-activated immunologic processes in systemic lupus erythematosus and their possible correction by interleukin 2. *Eur. J. Immunol.* 15:262.
17. Lattime, E. C., S. Gillis, C. David, and O. Stutman. 1981. Interleukin 2 production in the syngeneic mixed lymphocyte reaction. *Eur. J. Immunol.* 11:67.
18. Lattime, E. B., S. Gillis, G. Pecoraro, and O. Stutman. 1982. Ia-dependent interleukin 2 production in syngeneic cellular interactions. *J. Immunol.* 128:480.
19. Wolos, J. A., and J. B. Smith. 1982. Helper cells in the autologous mixed lymphocyte reaction. III. Production of helper factor(s) distinct from interleukin 2. *J. Exp. Med.* 156:1807.
20. Zubri, R., and A. Altman. 1982. Helper factor production in murine secondary mixed leukocyte reaction. *J. Immunol.* 128:817.
21. Suzuki, R., S. Suzuki, M. Igarashi, and K. Kumagai. 1986. Induction of interleukin 3 but not interleukin 2 or interferon production in the syngeneic mixed lymphocyte reaction. *J. Immunol.* 137:1564.

22. Arai, S., T. Munakata, K. Kuwano, K. Itoh, and K. Kumagai. 1984. Suppressive effect of human natural killer cells on pokeweed mitogen induced B cell differentiation. *J. Immunol.* 131:651.
23. Julius, M. H., E. Simpson, and H. Zenbarg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
24. Orosz, C. G., D. C. Roopenian, and F. H. Bach. 1983. Phorbol myristate acetate and in vitro T lymphocyte function. I. PMA may contaminate lymphokine preparations and can interfere with interleukin bioassay. *J. Immunol.* 130:1764.
25. Dexter, T. M., J. Garland, E. Scolnik, and D. Metcalf. 1980. Growth of factor dependent hemopoietic precursor cell line. *J. Exp. Med.* 152:1036.
26. Ihle, J. N., J. Keller, L. Henderson, F. Klein, and E. Palaszynski. 1982. Procedures for the purification of interleukin 3 to homogeneity. *J. Immunol.* 129:2431.
27. Itoh, K., K. Shiiba, Y. Shimizu, R. Suzuki, and K. Kumagai. 1985. Generation of activated killer (AK) cells by recombinant interleukin 2 (rIL2) in collaboration with interferon γ (IFN γ). *J. Immunol.* 130:3124.
28. Suzuki, R., K. Handa, K. Itoh, and K. Kumagai. 1983. Natural killer (NK) cells as a responder to interleukin 2 (IL2). I. Proliferative response and establishment of cloned cells. *J. Immunol.* 130:981.
29. Gillis, A. S., and K. A. Smith. 1977. Long term culture of tumor specific cytotoxic T cells. *Nature (Lond.)* 268:154.
30. Suzuki, R., S. Suzuki, N. Ebina, and K. Kumagai. 1985. Suppression of alloimmune cytotoxic T lymphocytes (CTL) generation by depletion of NK cells and restoration by interferon and/or interleukin 2. *J. Immunol.* 134:2139.
31. Itoh, K., A. B. Tilden, K. Kumagai, and C. M. Balch. 1985. Leu11⁺ lymphocytes with natural killer (NK) activity are precursors or recombinant interleukin 2 (rIL2)-induced activated killer (AK) cells. *J. Immunol.* 134:802.
32. Russo, C. F., F. Indiveri, V. Quanta, G. Molinaro, M. A. Pellegrino, and S. Ferrone. 1981. Stimulation of human T lymphocytes by PHA-activated autologous T lymphocytes: analysis of the role of Ia-like antigens with monoclonal antibodies. *Immunogenetics.* 12:267.
33. Romain, P. L., S. F. Schlossman, and E. L. Reinherz. 1984. Surface molecules involved in self-recognition and T cell activation in the autologous mixed lymphocyte reaction. *J. Immunol.* 133:1093.
34. Endo, Y., R. Suzuki, and K. Kumagai. 1986. Macrophage can produce factors capable of inducing decarboxylase, a histamine-forming enzyme, in vivo in the liver, spleen, and lung of mice. *Cell. Immunol.* 97:13.
35. Ihle, J. N., L. Rebar, J. Keller, J. C. Lee, and A. J. Hapel. 1982. Interleukin 3: possible roles in the regulation of lymphocyte differentiation and growth. *Immunol. Rev.* 63:5.
36. Zielske, J. V., and S. H. Golub. 1976. Fetal calf serum-induced blastogenic and cytotoxic responses of human lymphocytes. *Cancer Res.* 36:3842.
37. Misi, J., and T. A. Waldman. 1981. In vitro generation of antigen-specific hemolytic plaque-forming cells from human peripheral blood mononuclear cells. *J. Exp. Med.* 154:1069.
38. Naides, S. J., D. Redelman, and N. J. Zvaifler. 1985. The role of xenoantigen in the human autologous mixed lymphocyte reaction: Ability to respond in the absence of exposure to xenoantigen depends on the method of T-cell preparation. *Clin. Immunol. Immunopathol.* 34:216.
39. Farrar, W. L., H. M. Johnson, and J. J. Farrar. 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin 2. *J. Immunol.* 126:1120.

40. Smith, K. A. 1980. T-cell growth factor. *Immunol. Rev.* 51:337.
41. Kumagai, K., R. Suzuki, and T. Onta. 1984. Interleukin 3 (IL3): A hematopoiesis-regulating lymphokine. *Acta Haematol. Jpn.* 47:1635.
42. Spivak, J. L., R. R. L. Smith, and J. N. Ihle. 1985. Interleukin 3 promotes the in vitro proliferation of murine pluripotent hematopoietic stem cells. *J. Clin. Invest.* 76:1613.
43. Ihle, J. N., J. Keller, S. Oroszlan, L. E. Henderson, T. D. Copland, F. Fitch, M. B. Prystowsky, E. Goldwasser, J. W. Schrader, E. Palaszynski, M. Dy, and B. Lebel. 1983. Biological properties of homogeneous interleukin 3. I. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, P cell-stimulating factor activity, colony stimulating factor activity, and histamine-producing cell-stimulating factor activity. *J. Immunol.* 131:282.
44. Fung, M. C., A. J. Hapel, S. Ymer, D. R. Cohen, R. M. Johnson, H. D. Campbell, and I. G. Young. 1984. Molecular cloning of cDNA for murine interleukin-3. *Nature (Lond.)* 307:233.
45. Burgess, A. W., and D. Metcalf. 1980. The nature and action of granulocyte-macrophage colony stimulating factors. *Blood.* 56:947.
46. Gough, N. M., J. Gough, D. Metcalf, A. Kelso, D. Grail, N. A. Nicola, A. W. Burgess, and A. R. Dunn. 1984. Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte-macrophage colony stimulating factor. *Nature (Lond.)* 309:763.
47. Curtisinger, J. M., and D. P. Fan. 1984. Interleukin 3 augments the murine primary cytolytic T lymphocyte response to allogeneic tumor cells. *J. Immunol.* 133:267.
48. Palacios, R., G. Henson, J. Steinmetz, and J. P. Mckearon. 1984. Interleukin 3 supports growth of mouse pre-B-cell clones *in vitro*. *Nature (Lond.)* 309:126.
49. Yu, D. T. Y., N. Chiorazzi, and H. G. Kunkel. 1980. Helper factor derived from autologous lymphocyte. *Cell. Immunol.* 50:305.
50. Kasakura, S., M. Taguchi, Y. Watanabe, T. Okubo, T. Murachi, H. Uchino, and M. Hanaoka. 1983. Suppressor cell differentiation factor: A new mediator released by stimulated human lymphocytes and distinct from previously described lymphokines. *J. Immunol.* 130:2720.
51. Kasakura, S., M. Taguchi, T. Murachi, H. Uchino, and M. Hanaoka. 1983. A new mediator (suppressor cell induction factor) activating T cell-mediated suppression: Characterization of suppressor cells, kinetics of their generation, and mechanism of their action. *J. Immunol.* 131:2307.