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INTERLEUKIN 2 INDUCTION OF PROLIFERATION IN RESTING T LYMPHOCYTES REQUIRES CONTACT WITH MONOCYTES

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Abstract Resting human T cells are known to express significant numbers of intermediate but none or barely detectable low and high affinity IL-2 receptors (IL-2R). IL-2 alone failed to induce proliferation in these cells. However, in presence of small proportion of autologous monocytes, as low as 22 pM, IL-2 induced high levels of proliferation in resting T cells. Introduction of a semi permeable membrane between the two cell types or addition of an anti-CD11b mAb inhibited such induction of proliferation by IL-2. Neither recombinant IL-1 nor IL-1-containing cell-free extracts from activated monocytes substituted for intact monocytes. Autologous B cells failed to replace monocytes. Using antigen-specific cloned human T cells we have shown a lack of requirement for antigen. The proliferation was inhibited by anti-IL-2Rα mAb. IL-2 appears to be unique since neither IL-4 nor IL-6, alone or in presence of monocytes, led to induction of proliferation in resting T cells. Combination of IL-2 and monocytes induced proliferation in all T cell subpopulations ($CD4^+$, $CD8^+$, $CD45RA^+$ and $CD45RO^+$) and antigen-specific clones examined. It also induces mRNA and surface expression of IL-2Rα, appearance of high affinity IL-2R and induction of proliferation in large proportions of T cells. As in humans, the IL-2 induction of proliferation in murine resting T cells required contact with syngeneic monocytes, suggesting that such a mechanism of T cells activation is highly conserved.

Resumen ***La proliferación de linfocitos T en reposo inducida por interleuquina 2 requiere del contacto con monocitos.*** Los linfocitos T (LiT) humanos en estado de reposo expresan un número significativo de receptores para IL-2 (IL-2R) con afinidad intermedia pero prácticamente no se detectan con baja y alta afinidad. IL-2 solamente induce proliferación de LiT en reposo en presencia de una pequeña proporción de monocitos autólogos. La proliferación requiere del contacto entre estos dos tipos celulares, ya que es inhibida por la presencia de una membrana semi-permeable o por anticuerpos anti-CD11b. Los monocitos no pueden ser reemplazados ni por linfocitos B autólogos ni sustituidos por el agregado de IL-1 recombinante o proveniente de extractos de monocitos activados. La falta de requerimiento antigénico en este proceso fue demostrada utilizando clones de LiT. La proliferación fue inhibida con Acm contra IL-2Rα. Citocinas como IL-4 o IL-6, solas o en presencia de monocitos, fueron incapaces de inducir la proliferación de LiT en reposo. La combinación de IL-2 y monocitos produjo la proliferación de todas las subpoblaciones de LiT ($CD4^+$, $CD8^+$, $CD45RA^+$ y $CD45RO^+$) al igual que clones específicos de antígeno. Esto también indujo la producción de ARNm y expresión de IL-2Rα, la aparición de IL-2R de alta afinidad y la proliferación de un gran número de LiT. Un mecanismo de activación similar ocurre en ratones, ya que la proliferación de células T murinas en reposo es dependiente de la presencia de IL-2 y contacto con monocitos. Esto sugiere que el mecanismo de activación de LiT se encuentra altamente conservado.

Key words: T lymphocytes, IL-2, IL-2 receptors, monocytes

Antigen-specific clonal expansion of T cells relies on successive and distinct receptor-ligand interactions. Antigen-presenting cells (APC) provide T cells not only with an antigen-specific stimulatory signal (ligation of the TCR) and a series of co-stimulatory signals (ligation of CD28, LFA1 and many other surface molecules) but also with polarizing signals (secretion of IL-12, IL-18, PGE2). Those interactions serve to select the T cells capable of responding to the antigen and trigger a series of biochemical changes in T cells leading to induction of IL-2 receptors (IL-2R) and, in some cases, of IL-2. Binding of IL-2 to its newly acquired receptors on T cells, leads to blastogenesis and entry of T cells into S phase[1].

The IL-2R were originally classified into three isoforms, the high-, intermediate-, and low-affinity receptors[2]. The work done during the last fifteen years has allowed the molecular characterization of the three

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distinct subunits (α, β and γ) that constitute IL-2R complexes. Expression of IL-2Rα alone or of both IL-2Rα and γ shows low affini-ties (k_d~10^{-8}M) to IL-2 binding, the βγ heterodimer complex exhibites intermediate affinities (k_d~0.5-1.5x10^{-9}M) and the αβγ heterotrimer complex binds to IL-2 at still higher affinity (k_d~10^{-11}M)[3].

IL-2Rγ is expressed on all human leukocytes, IL-2Rα and β are differentially expressed on lymphocytes subpopulation, although their expressions are enhanced by antigens and mitogens. $CD8^+$ T and NK cells express IL-2Rβ, but little of IL-2Rα, while $CD4^+$ T cells express faint amounts of IL-2Rβ[4].

However, IL-2 itself is shown to induce proliferation in a sizeable proportion of resting human T cells, apparently bypassing the requirement of antigen[5-9]. This intriguing phenomenon was first reported several years ago but its mechanism remained unknown. This is because the essential requirements of such proliferation have not been clearly established as several results of the studies of this process are controversial. Some studies attributed such proliferation to a direct action of IL-2 on resting T cells through IL-2Rβ[5, 7] whereas others[6, 8, 9] including our own[10] demonstrated a requirement of monocytes. In some of these studies, IL-1 was shown to replace monocytes in this process. This implied a direct action of IL-1 on resting T cells. However, resting T cells, unlike activated cells, express barely detectable IL-1 receptors[11]. The induction of proliferation is accompanied by the induction of IL-2Rα and blocked by anti-IL-2Rα mAbs[5, 8]. However, the requirements for induction of IL-2Rα are unclear and the T cells preparations used in this study proliferated in response to Con A and hence were not pure.

In order to understand its mechanism and possible physiological significance, we decided to determine the key requirements of IL2-induced proliferation in resting T cells. We demonstrate that IL-2 in the presence of monocytes, but not alone, induces the expression of both IL-2Rα and high affinity IL-2 receptors in T cells and then causes their proliferation. The induction of proliferation occurs irrespective of lineage and specificity of the T cell.

Materials and Methods

Interleukins: Recombinants human IL-1 were purchased from Collaborative Research Inc. Recombinant human IL-2 (1 unit/ml = 22 pM) was procured from the Cetus Corporation. ^{125}I-labelled recombinant human IL-2 (38 µCi/µg) was bought from the New England Nuclear. The recombinant human IL-4 and IL-6 were purchased from Genzyme.

Antibodies and flow cytometric analysis: Purified rabbit anti-human-IL-2 antibody was purchased from Genzyme. An anti-Tac mAb was a gift from Dr. Waldmann. The anti-DR mAb 7H.3[12], anti-human B cell mAb 41H16[13], and mAb 49H.8 that binds to mouse NK cells[14] were provided by Dr. Longenecker. The anti-CD45RO mAb UCHL1[15] and anti-CD45RA mAb 3AC5[16] were gifts from Dr. Beverley and Dr. Ledbetter, respectively. The hybridoma (M7/20) against mouse high affinity IL2 receptor[17] was received from Dr. Strom. Hybridomas producing OKT3, OKT4, OKT8, anti-OKM1, anti-MMA, anti-HLA-DR (L243), anti-IA^d (MK-D6), or anti-mouse monocytes (F4/80) mAb were obtained from ATCC. Purified goat anti-mouse-Ig was purchased from Cappel Laboratories. Anti-Tac, anti-OKM1, and 49H.8 mAbs were used after purification on Protein A-Sepharose columns. For analysis on FACS, cells were stained with appropriate mouse mAb and then with FITC-conjugated goat anti-mouse antibody, fixed with 1% paraformaldehyde and analyzed on a cytometer, as described[18].

PBMC and T cell purification: PBMC were isolated by Ficoll-Hypaque density gradient centrifugation of freshly drawn heparizinzed blood or buffy coat. For purification of T cells, the blood sample was first incubated (45 min, 37°C) with carbonyl iron and then subjected to centrifugation on Ficoll-Hypaque. The layer of mononuclear cells was recovered and cells adherent to plastic or Sephadex G10 removed. The non-adherent cells were incubated (45 min, 4°C) with a mixture of mAbs (anti-DR, anti-OKM1, anti-Tac, and anti-B cell; unless stated otherwise) washed and treated (40 min, 37°C) with Low-Tox-H rabbit C (Cerdarlane). The recovered cells were 99.5% $CD2^+$ and ~95% $CD3^+$ and failed to proliferate in response to Con A (Sigma) or PHA (GIBCO) unless supplemented with monocytes. These are referred to as purified T cells. The T cells were treated with OKT8 or OKT4 plus C to obtain $CD4^+$ or $CD8^+$ T cell subpopulations, respectively. Total or $CD4^+$ T cells were treated with either mAb UCHL1 or mAb 3AC5 and then with C to obtain $CD45RA^+$ or $CD45RO^+$ T cell subpopulation, respectively[18]. In some experiments, the antibody bound cells were removed by attachment to anti-mouse-IgG antibody-coated magnetic beads (Dynabeads; Dynal, Great Neck, New York) rather than by lysis.

Human monocytes and B cells: PBMC were suspended (1x10^7/ml) in RPMI 1640 supplemented with 10% autologous serum (heat inactivated), and incubated (90 min, 37°C) in a plastic tissue culture petri-dish. The dish was subjected to rotary motion and the non-adherent cells removed by aspiration and, if desired, used for B isolation. This step was repeated four times. The adherent monocytes were detached by scraping with a soft plastic tip and consisted of > 95% alpha-naphthyl acetate esterase positive cells. When required, monocytes were activated by LPS. For cell-free extracts, 2x10^6 activated monocytes were lysed in 1 ml PBS by three cycles of quick freeze and thaw. The lysate was centrifuged at 5 000xg and the supernatant containing high levels of IL-1 activity[19] used as monocyte extract. B cells were isolated from monocyte-depleted E^- (SRBC non-rosetting) PBMC by panning on anti-human-Ig antibody-coated culture plates.

Mouse T cells and monocytes: Lymph node cells from 6 to 10-week old Balb/c mice were suspended (x10^6/ml) in RPMI 1640 - 10% FCS) and incubated (90 min, 37°C) in a plastic tissue culture dish. The non-adherent cells were recovered, 5x10^7 of them resuspended in 1 ml PBS, and passed at flow rate of pre-swollen Sephadex G10 (2ml) in PBS. The unbound cells were resuspended (1x10^7/ml) in 4°C BSS-BSA (1% BSA in basic salt solution) containing anti-IA^d (MK-D6), anti-IL2 receptor (M7/20), anti-monocytes (F4/80), anti-mouse-Ig, and anti NK cell (49H.8) mAbs. Following incubation (40 min, 4°C), cells were washed and treated (30 min, 37°C) with C. The cells recovered were ~98% $CD3^+$ and did not proliferate in response to Con A unless supplemetned with monocytes. For isolation of monocytes, the spleen cells from the same or syngeneic mice were suspended (1x10^7/ml) in FCS-medium and incubated in a petri-dish, as for isolation of human monocytes. The non-

adherent cells were discarded and adherent cells rcovered as described above.

Cell culture and DNA synthesis: Unless stated otherwise, cells were cultured in RPMI 1640 - 10% autologous serum and incubated at 37°C with 7% CO_2 and 90% humidity. Routinely, 0.8 to 1x10^5 T cells were cultured in 0.2 ml medium in flat bottom 96-well plates in the presence of 5 to 10% autologous irradiated (3 000 rads) monocytes and IL-2. Monocytes isolated in the presence of autologous serum were used to avoid the introduction of exogenous antigens in the culture. Rate of DNA synthesis was measured on day 6 during a four-hour pulse with ^{3}H-thymidine (1µCi/well; 80Ci/mmole; NEN). Then values presented are average of three or four replicates which, unless indicated otherwise, varied within a span of 15% of the average.

Antigen-specific T cell clones: Human monoclonal T cell lines, specific for either purified protein derivative of tuberculin (PPD) or tetanus toxoid or keyhole limpet haemocyanin, were derived from a donor who was immunized with first two antigens. 1x10^6 PBMC were cultured in the presence of 50µg/ml antigen. On day 6, 80% of the medium was replaced with fresh medium containing the antigen and 3x10^5 irradiated (3 000 rads) autologous PBMC. On day 9, IL-2 (final concentration 0.25 nM) was added. On days 12 and 18, the medium, antigen and irradiated PBMC were replenished, and on days 15 and 21 IL2 re-added, as describe above. On day 22, the live cells from culture were separated by centrifugation on Ficoll-Hypaque and cloned by limiting dilution, as described[20]. The cloned T cells were maintained in culture by weekly feeding.

IL-2Rα and high affinity IL2 receptors: The presence of IL-2 (free or cell-bound) interfered with binding of both anti-Tac (IL-2Rα) mAb and ^{125}I-IL2. Therefore, prior to assay, the cells were washed and the pre-existing IL-2/IL-2 receptor complexes minimized by incubation (60 min, 37°C; twice) and washings, as described[8]. The IL-2Rα expression was monitored by flow cytometry after staining with anti-Tac mAb. The high affinity IL2 bindings sites were assayed as described previously[10]. The binding mixture (in 0.5 ml FCS-medium) contained ~4.5x10^6 cells, 2 mg/ml BSA and 100 pM ^{125}I-IL2, and was incubated at 37°C for 20 min. The radioactivity bound to cells was determined in quadruplet after separation from free ^{125}I-IL2 by centrifugation (2 min, 13 000xg) through a silicon oil mixture. The non-specific ^{125}I-IL2 binding was determined in parallel assays in the presence of a 100-fold excess of unlabelled IL2- and was subtracted to obtain specific IL-2 binding.

Results

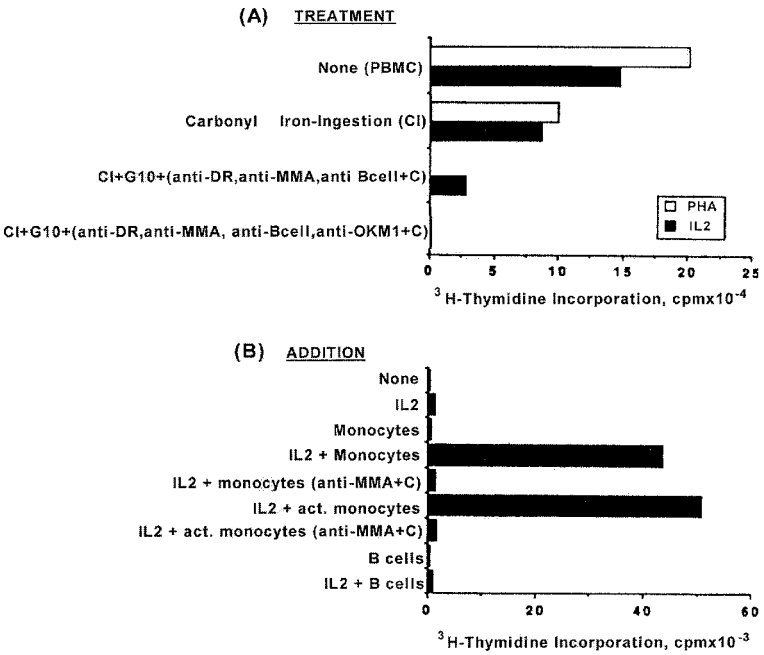

Fig. 1. *IL2-induction of proliferation in resting T cells is monocyte-dependent.* (A) PBMC were treated, as indicated, to obtain T cell populations with varying proportions of monocytes and NK cells. 8x10^4 cells were cultured in medium containing 10% autologous serum in the presence of either 1% PHA or 11 nM IL2, and ^{3}H-thymidine incorporation monitored on day 3 or 6, respectively. The incorporation in cultures without PHA and IL 2 was < 1 000 cpm. (B) 10^5 purified T cells were cultured with the indicated addition. The concentrations were 1.1 nM IL2 and 10^4 autologous irradiated (3 000 rads) either monocytes (with or without activation with LPS) or B cells per culture. ^{3}H-thymidine incorporation was monitored on day 6. The incorporations by either monocytes or B cells, with or without IL2, were < 600 cpm. In both (A) and (B), the results are typical of several experiments performed.

IL-2 induction of proliferation in resting human T cells requires monocytes: Addition of IL-2 to cultures of PBMC induces high levels of proliferation in apparent absence of any nonself stimulus[8, 10]. The proliferation can be monitored by DNA synthesis which shows a peak on day 6. Fig. 1A shows levels of IL-2 induced proliferation as a function of monocytes depletion. Monocyte-depleted PBMC (CI-treatment) exhibited lower proliferation in response to IL-2 or PHA than total PBMC. Further purification of T cells by removal of Sephadex-adherent cells and of DR^+, MMA^+ and B cells eliminated any proliferation to PHA. These cells still exhibited low but significant proliferation in response to IL-2. Removal of $OKM1^+$ cells in addition to the above cells completely abolished any proliferation due to IL-2. Since OKM1 is expressed on both NK cells and monocytes[22, 23] and MMA only on the latter[24], the proliferation in MMA^-OKM1^+ cells is likely due to NK cells. The purified T cells did not proliferate despite the presence of 11 nM IL-2 which is far more than sufficient for binding and signaling via IL-2Rβγ[6, 7].

Addition of autologous monocytes (1-10%) restored the IL2-induction of proliferation in purified resting T cells (Fig. 1B). Treatment on monocyte preparations with anti-MMA plus C abolished their ability to restore IL2-induction of proliferation in resting T cells. Activated monocytes were only slightly more effective than untreated monocytes. In contrast to monocytes, addition of similar concentrations of autologous B cells failed to restore the induction of proliferation in resting T cells by IL-2.

Low concentrations of IL-2 is sufficient for induction of proliferation in T cells and its subpopulations: Resting T cells express 400 to 700 IL-2Rβ/cell but undetectable high affinity receptors, as indicated by measurement of radioactivity and autoradiography (our unpublished results) after binding of ^{125}I-IL2. Results in Fig. 2A show that, in presence of autologous monocytes, as low as 1 unit/ml or 22 pM IL-2 was sufficient to induce significant proliferation in resting T cells. Addition of anti-IL2 antibody at the beginning of cultures blocked the induction of proliferation in an antibody-concentration dependent manner. From these results we conclude that, in presence of monocytes, the concentrations (such as 22 and 44 pM) of IL-2 partially saturating for binding to high affinity receptors but not to IL-2Rβ are sufficient to induce proliferation in resting T cells. The proliferative response rose linearly with increasing concentration of IL-2, without noticeable change in the slope during transition from 22 and 44 pM to 660 and 2 200 pM (saturating for binding to both high affinity receptors and IL-2Rβ).

In presence of monocytes, low concentrations of IL-2 were able to induce direct proliferation in $CD4^+$, $CD8^+$, $CD45RA^+$, $CD45R0^+$ T cells (not shown). Thus, induction of proliferation by IL-2 and monocytes appears be a common property of most T cells and not that of a unique subset (also see below results of T cell clones).

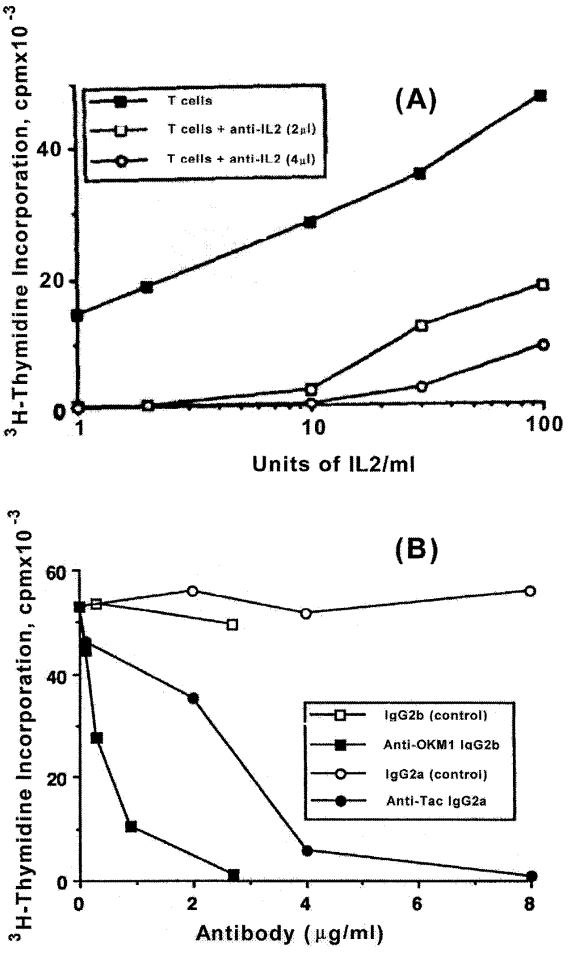

Fig. 2.– *IL2-induction or proliferation in T cells.* (A) 10^5 total T cells were cultured in presence of 10^4 autologous monocytes (irradiated) and the indicated concentrations of IL2 (1unit/ml = 22pM). Cultures contained either 2 µl/ml or 4 µl/ml of anti-IL2 antibody. 1 µl antibody neutralized 10 units of IL2 dissolved in 1 ml FCS-medium. (B) 10^5 purified T cells were cultured in the presence of 0.22 nM IL2, 10^4 autologous (irradiated) monocytes and the indicated concentrations of a purified mAb. The ^{3}H-thymidine incorporation was monitored on day 6.

Inhibition by antibody blocking of CD11b or IL-2Rα: OKM1 is an epitope on CD11b molecule which is expressed on monocytes but not T cells[22]. The IL-2Rα is barely detectable on peripheral blood monocytes[27] and resting T cell[4] but induced in each upon activation. In an initial attempt to determine the roles for these molecules, the effects of specific mAbs that bind to them were examined. Fig. 2B shows that the addition of anti-OKM1

TABLE 1.– *IL4 OR IL6 fails to substitute for IL2*

Addition	^{3}H-Thymydine incorporation (CPM)				
	T	CD4$^+$	CD8$^+$	CD45RA$^+$	CD45RO$^+$
Monocytes	470	870	548	428	305
IL2	1 073	3 135	2 034	2 160	1 890
Monocytes + IL 2	29 132	49 761	33 112	35 894	27 430
IL4	625	679	478	754	456
Monocytes + IL 4	976	632	594	389	403
IL6	295	ND	ND	ND	ND
Monocytes + IL 6	347	ND	ND	ND	ND

8 x 10^4 total cell or their subpopulation (< 98% cells expressing subpopulation specific marker) were cultured with or without 10^4 autologous (irradiated) monocytes. Where indicated, the cultures contained 1.1 nM IL2 or 200 µ/ml IL4 or 500 µM/ml IL6. The ^{3}H-Thymidine incorporation was measured on day 6. The incorporation by monocytes without any lymphokine was < 360 cpm.

or of anti-IL-2Rα mAb at the beginning of the cultures inhibited the IL2-induction of proliferation in resting T cells, in an antibody-concentration dependent manner. This suggests that both CD11b and IL-2Rα are parts of the mechanism of monocyte-dependent IL-2-induction of proliferation in resting T cells.

Neither IL-4 nor IL-6 substitutes for IL-2: In addition to IL-2, IL-4 and IL-6 are T cell growth factors which also induce proliferation and differentiation in B cells[25]. As shown in Table 1, neither IL-4 (200 µ/ml) nor IL-6 (500 µ/ml) alone induced any significant proliferation in resting T cells. However, unlike IL-2, these lymphokines also failed to induce proliferation in presence of monocytes. The biological activities of IL-4 and IL-6 were confirmed in parallel assays by monitoring the induction of proliferation and differentiation in B cells (not shown). The results suggest that IL-2 may be unique in its ability to induce a monocyte-dependent proliferation in resting T cells.

IL-1 and monocyte extracts do not substitute for monocytes: IL-1 is intricately implicated in T cell activation[19, 26]. Previous studies have shown that IL-1 or IL-1-like activity produced by LPS-activated monocytes can replace monocytes in IL-2-induction of proliferation in T cells. However, since resting T cells express very low levels of IL-1 receptors, we re-examined the ability of IL-1 to mediate this process. Results in Table 2 show that recombinants human IL-1 failed to substitute for monocytes in IL-2-induction of proliferation in resting T cell. The biological activity of IL-1 preparations was confirmed in a parallel IL-1 bioassay (Table 2 lower section). Cell-free crude extracts from LPS-activated monocytes also failed to replace monocytes. This showed that IL-1, either alone or in the presence of some soluble factors from activated monocytes, is insufficient to substitute for intact monocytes in IL-2-induction of proliferation in resting T cells.

TABLE 2.– *IL1 or monocyte extract fails to replace monocytes*

Addition to T cells	^{3}H-Thimidine incorporation (CPM) Experiment 1	Experiment 2
None	317	257
IL1 (alpha-beta)	ND	304
IL2	1 438	1 131
Monocytes	795	504
IL2+Monocytes	57 549	31 422
IL2+IL1 (alpha)	2 684	ND
IL2+IL1 (beta)	3 021	ND
IL2+IL1 (alpha+beta)	3 521	2 143
IL2+Monocyte extract	3 478	ND

Addition to indicator T cells	IL2 production (units/ml)
None	0.51 ± 0.07
PHA	0.99 ± 0.10
IL1 (alpha+beta)	0.33 ± 0.12
PHA+IL1 (alpha)	14.75 ± 1.18
PHA+IL1 (beta)	12.96 ± 0.80
PHA+IL1 (alpya-beta)	12.99 ± 2.01

Upper section: *replicates of 10^5 T cells were cultured with indicated additions. Their concentrations were 20 µ/ml recombinant IL1 alpha or beta, 20 µ/ml each recombinant IL1 alpha plus beta, 1.1 nM IL2, 10^4 autologous (irradiated) monocytes per culture, and cell-free extract from 2x10^4 LPS-activated monocytes per culture. The preparation of the extract is described in Materials and Methods.*
Lower section: *The IL1 biossay was based upon the ability to complement pHA in inducing IL2 production from a mouse splenic lymphoma T cell line LBRM-33-1A5[25]. The IL1 concentration tested was 20 µ/ml. IL2 production was monitored by CTLL proliferation assay. One unit IL2 led to incorporation of 2x10^6 cpm of ^{3}H-Thymidine in 10^4 CTLL cells in 16 hours.*

IL-2-induction of proliferation occurs independently of antigen: IL-2 in combination of monocytes induces proliferation in resting T cells in apparent absence of any nonself stimulus. For a conclusive demonstration of a

lack of requirement for the antigen, we examined the response of several antigen-specific human T cell clones. The cells used were specific for PPD or tetanus toxoid or keyhole limpet haemocyanin. The T cells from each clone were passed through a Sephadex G10 column to remove residual monocytes and then cultured in the absence of feeder cells and antigen but in presence of IL-2. After 7 days, IL-2Rα expression on cells had declined considerably and they exhibited only low levels of DNA synthesis in response to IL-2. At this stage, the T cells were washed and tested for proliferation in response to IL-2 and monocytes. T cell from all the nine clones tested underwent vigorous proliferation in the presence of IL-2 and monocytes. The results of one of the PPD-specific clones depicted in Fig. 3 are representative of others. The PPD-specific T cells failed to proliferate in response to either antigen or monocytes alone but showed little proliferation in response to IL-2. The latter was apparently due to a few residual high affinity IL-2 receptors on T cells. However, high levels of proliferation were induced by incubation of T cells in presence of either antigen plus monocytes or IL-2 plus monocytes, the latter combination causing much grater proliferation that the former. These

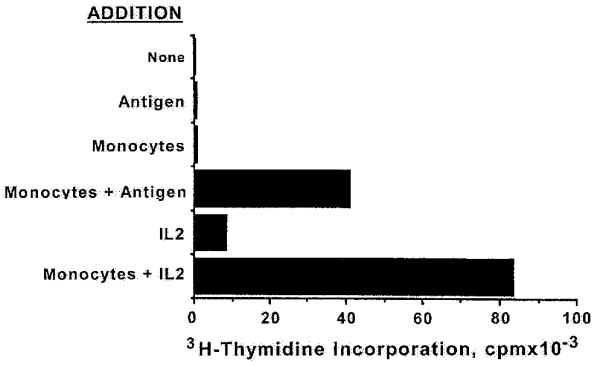

Fig. 3.– *IL2-induction of proliferation in T cells is antigen-independent.* Five days after feeding, live cells from a culture of PPD-specific human T cell clone were recovered by centrifugation on Ficoll-Hypaque. The cells were washed, passed trough a Sephadex G10 column and the non-adhering cells cultured in FCS-medium in the presence of 1.1 nM IL2. On day 7, cells had lost over 90% of the responsiveness to IL2. At this stage, cells were washed and replicates of $8x10^4$ cell cultured in 0.2 ml FCS-medium in the presence of medium alone (none) or 50 µg/ml PPD or 1.1 nM IL2 or $8x10^3$ autologous (irradiated) monocytes or the indicated combination of the two. ^{3}H-thymidine incorporation was measured on day 4.

TABLE 3.– *A requirement for T cell contact with monocytes*

Top	Bottom	^{3}H-Thymidine incorporation (CPM ± S.D)
–	T cells + IL2	1 437 ± 291
–	T cells + monocytes	1 101 ± 295
–	T cells + monocytes + IL2	38 371 ± 5 262
IL2	T cells + monocytes	29 003 ± 3 013
IL2 + monocytes	T cells	2 132 ± 612
IL2 + T cells	monocytes	3 028 ± 851
–	$CD4^+$T cells + IL2	2 209 ± 691
–	$CD4^+$ T cells + IL2	56 363 ± 4 422
IL2	$CD4^+$ T cells + monocytes	53 168 ± 8 720
IL2 + monocytes	$CD4^+$ T cells	2 922 ± 1 092
–	$CD8^+$ T cells + IL2	2 915 ± 943
–	$CD8^+$ T cells + monocytes + IL2	33 956 ± 3 509
IL2	$CD8^+$ T cells + monocytes	27 015 ± 1 178
IL2 + monocytes	$CD8^+$ T cells	1 593 ± 181
–	mouse T cells	313 ± 87
–	mouse T cells + IL2	2 675 ± 277
–	mouse T cells + monocytes + IL2	23 100 ± 1 814
IL2	mouse T cells + monocytes	17 864 ± 2 270
IL2 + monocytes	mouse T cells	1 758 ± 131
IL2 + mouse T cells	monocytes	1 856 ± 156

Cells were cultured in 24-well plates. Each well was partitioned into top and bottom compartments by introduction for a 0.45 µ membrane at its bottom (manufactured by Costar). 10^6 total or ($CD4^+$ or $CD8^+$) subpopulation of T cells were cultured in 2 ml medium in the presence or absence of 1.2-1.6 x 10^5 autologous (irradiated) monocytes and/or 1.1 nM IL2, as indicated. On day 5.5, cells from the two compartments were mixed together and four replicates of 105 µl culture pulsed for six hours with 1 µCi of ^{3}H-Thymidine

results demonstrate that induction of proliferation by combination of IL-2 and monocytes is an antigen-independent process and it occurs irrespective of the clonal specificity of the T cells.

Requirement of contact between T cells and monocytes: The inability of IL-1 and IL-1-containing monocyte extracts to substitute for monocytes suggested the possibility for a role of monocyte surface molecules in IL-2-induction of proliferation in resting T cells. To test this idea, we introduced a 0.45 µ membrane between T cells and monocytes. The membrane prevented any contact between two types of cells but allowed flow of macromolecules through its pores. As a control for the flow of the molecules across the membrane, IL-2 was added on one side (top) of the membrane and both T cells and monocytes on the other (bottom), and proliferation of T cells monitored after the incubation. Results in Table 3 show that combination of IL-2 and monocytes induced proliferation in T cells regardless of whether IL-2 was added to the side of membrane containing T cells and monocytes or across the membrane. However, when T cells and monocytes were separated by the membrane, IL-2 was unable to induce a similar proliferation. These results show a requirement for contact between T cells and monocytes. Such contact was necessary for IL-2-induction of proliferation in both $CD4^+$ and $CD8^+$ subpopulations of T cells. Similar results were obtained using highly purified resting mouse T cells (Table 3).

Induction of IL-2Rα and high affinity IL-2 receptors: Previous studies have shown that IL-2 alone was sufficient to induce IL-2Rα and proliferation of resting T cells[5]. Our results so far do not agree with this claim since we have shown that IL-2 alone is unable to induce IL-2Rα mRNA in resting T cells wich are rigorously depleted of monocytes[10]. The surface expression of IL-2Rα, determined by flow cytometry after staining with an anti-IL-2Rα mAb, indicate that the combination of IL-2 and monocytes induce high levels of IL-2Rα in a large proportion (30 to 40%) of T cells two days prior to the peak of DNA synthesis (not shown). The high affinity IL-2 receptor was monitored by binding in the presence of 100 pM ^{125}I-IL2. Fig. 4 shows that a large number of high affinity IL-2 binding sites were induced in T cells that were cultured in the presence of IL-2 plus monocytes but not in those cultured with either IL-2 or monocytes. The appearance of high affinity receptors therefore accompanied the expression of IL-2Rα.

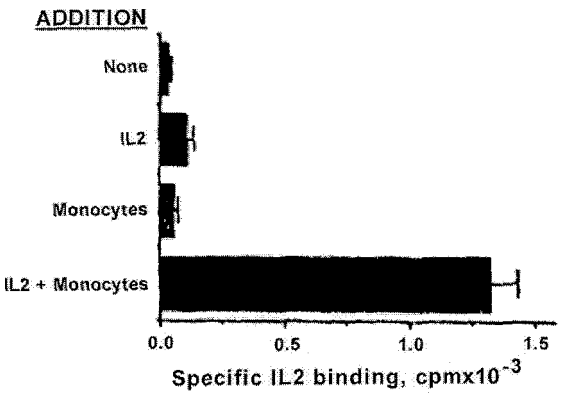

Fig. 4.– *Induction of high affinity IL2 receptor.* Purified T cells were cultured in the presence of medium or 1.1 nM IL2 or 10% autologous monocytes or 1.1 nM IL2 plus 10% autologous monocytes. On day 6, the high affinity IL2 binding sites were estimated in the presence of 100 pM ^{125}I-IL2, as described in Materials and Methods. The results shown are specific IL2 per 10^6 cells.

Discussion

Resting T cells express significant numbers of IL-2Rβ but barely detectable IL-2Rα[5, 6]. Treatment of these cells with IL-2, even at the concentrations that are partially saturating for binding to IL-2Rβ, fails to induce either IL-2Rα[10] or proliferation (Fig. 1). As much as 0.22 µM IL-2 was unable to induce proliferation in these cells (unpublished results). However, unlike resting T cells, the NK cells present in OKM1+MMA-DR-PBMC proliferated in direct response to IL-2 (Fig. 1A). This was in agreement with findings of Ben Aribia et al.[6] who showed that, despite the similar numbers of IL-2Rβ in IL-2Rα- NK and resting T cells, IL-2 alone induces proliferation in former but not in the latter cells. This difference may in part be due to the internalization of IL-2Rβ-bound to IL-2 in NK cells but not in resting T cells[7, 28].

In the presense of a small proportion (1 to 10%) of autologous monocytes, IL-2 induces the expression of both IL-2Rα mRNA[10] and high affinity IL-2 receptors in resting T cells (Fig. 4). Subsequent interactions between IL-2 and its high affinity receptors, as expected[28], leads to induction of proliferation in T cells (Fig. 1B). The induction is inhibited by anti-IL-2Rα mAbs ([5, 8]; and Fig. 2B). The overall process requires low concentrations of IL-2 that are partially saturating for binding to the high affinity receptors but not to IL-2Rβ (Fig. 2A). Since both peripheral blood monocytes[27] and resting T cell[6] express barely detectable IL-2Rα (hence none or barely detectable high affinity IL-2 receptors), the target cell for the initial action of IL-2 remains unknown. It is possible that during IL-2-induction of IL-2Rα in T cells, the IL-2 first acts on monocytes and induces IL-1 which then mediates the induction of IL-2Rα in T cells[26]. A direct action of IL-2 on resting T cells, via a small number of undetectable high affinity receptors, which fails to induce proliferation but established communications with monocytes cannot be ruled out.

Neither recombinant IL-1 nor IL-1-containing cell-free extracts from activated monocytes replaced monocyte

requirement during IL-2-induction of proliferation in resting T cells (Table 2). This was not surprising in view of the fact that resting T cells express only very low levels of IL-1 receptors[11]. The observation is, on the other hand, consistent with our finding that the induction of proliferation required contact between T cells and monocytes (Table 3). We are in the process of identification of surface molecules that establish the cell contact and transduce activation signal in T cells. Since the contact and the induction of IL-2Rα or proliferation (Fig. 3) occur independently of antigen, the precise nature of the T cell contact with monocytes is expected to be different from that during antigen recognition[1]. There are at least two known surface interactions, other than with CD3/TCR, that lead to a non-clonotypic activation of T cells. One involves triggering through CD2 by its natural ligand LFA-3[29] and the other through CD28[30]. Some of these molecules are also involved in cell adhesion and therefore play a role during antigen-dependent T cell activation[1]. We examined the effect of antibody-blocking of one of the components of leukocyte adhesion molecule, CD11b, which is expressed on monocytes but not on T cell. Antibodies against CD11b are known to inhibit several monocyte functions including adhesion and aggregation but do not normally affect T cell proliferation[31]. However, we found that anti-OKM1 mAb that specially binds to CD11b sharply inhibited IL-2-induction of proliferation in T cells (Fig. 2B). It is possible that CD11b is involved in the contact between monocytes and resting T cells. The antibody-blocking of CD11b, in such a case, will be equivalent to having a membrane between T cells and monocytes, as in Table 3. Alternatively, the binding of anti-OKM1 to CD11b may generate a signal which inhibits the release of unidentified molecules from monocytes that mediate the activation of T cells. An anti-DR framework mAb was also shown to block IL-2-induction of proliferation in resting T cells[32]. We reproduced this result but then found that this mAb is able to inhibit proliferation directly in both $CD4^+$ and $CD8^+$ T cells (unpublished results). Since induction of proliferation in both human and mouse T cells (Table 3) required contact with monocytes, the molecules involved in making the contact may be related if not conserved.

Previous studies have shown that IL-1 can partially substitute for monocytes[8]. We believe this is due to a partial activation of T cell during their purification or to presence of contaminating monocytes in T cells preparations. We have observed that IL-1 or IL-1-containing extracts from activated monocytes, which fail to substitute for monocytes during IL-2-induction of proliferation in resting T cells, can partially replace the monocyte requirement IL-2-induction of proliferation in previously activated quiescent T cells (unpublished results). This difference is likely due to the presence of residual IL-1 receptors on some of previously activated T cells but not on resting T cells. It is possible that, in presence of IL-2, the contaminating monocytes in T cells may allow a partial induction of IL-1 receptors on resting T cells. The addition of IL-1 under these conditions will produce IL-1-dependent effects normally not seen in monocyte-free resting T cells. The above results nevertheless suggest a role for IL-1 in IL-2-induction of proliferation in resting T cells. A plausible mechanism for IL-2-induction of proliferation may, therefore, first involve induction of IL-1 receptors on resting T cells by combination of IL-2 and monocytes and induction of IL-1 production by monocytes in response to IL-2. The former may require contact between T cells and monocytes. Interactions between IL-1 and its receptor on T cells may lead to induction of IL-2Rα, as previously shown in cloned T and NK cells lines[26, 33]. The association of newly synthesized IL-2Rα with IL-2Rβ (either pre-existing or also newly synthesized) will result in appearance of high affinity receptors, enabling the T cells to internalize IL-2[28] and proliferate in response to IL-2.

The combination of IL-2 and monocytes is directly able to induce proliferation in all T cell subpopulations and clones examined (Fig. 2, 3, Table 3). The combination induced IL-2Rα expression in at least 20% of T cells under conditions in which DNA synthesis was pharmacologically inhibited (unpublished data). This therefore represents a major endogenous mechanism of T cell activation. As indicated by its requirement, it is distinct from other well-known mechanisms[29, 30]. Such antigen-independent mechanism of proliferation may be involved in the formation[34] and maintenance[35] of T cell repertoire in vivo. The mechanism is operative in both man and mouse and therefore appears to be conserved.

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