Monocyte Interleukin-1 Secretion Is Regulated by the Sequential Action of γ-Interferon and Interleukin-2 Involving Monocyte Surface Expression of Interleukin-2 Receptors *

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A. Introduction

Interleukin-1 (IL-1) is a polypeptide synthesized as a high-mol.-wt. precursor and subsequently secreted after proteolytic cleavage to 17 500-dalton active forms in murine [1] and human cells [2]. Recently, cDNAs for murine [3] and for two distinct human IL-1 species, IL-1α and IL-1β, have been isolated, sequenced, and cloned [2, 4].

IL-1 is produced by a variety of cell sources including macrophage-containing tissues, such as peripheral blood, bone marrow, dendritic cells, pulmonary alveolar cells, Kupffer cells, astrocytes, and glial cells (reviewed in [5, 6]) and non-macrophage cells such as B-lymphocytes [7], large granular lymphocytes [8], epidermal cells [9], and mesangial cells [10], and it exerts a multiplicity of nonspecific biological activities amplifying inflammatory reactions and modulating the immune response. IL-1 activity results in the release of acute phase reactants such as serum amyloid P component, fibrinogen and C-reactive protein [11, 12], increases the production of collagenase and prostaglandins by rheumatoid synovial cells and chondrocytes [13], induces synthesis of prostacyclin [14] and procoagulant activity [15] in vascular cells, acts on vascular endothelial cells to increase adhesion of granulocytes and monocytes [16], stimulates fibroblast growth [17], release of γ-interferon (IFN) [18], and osteoclast-mediated bone reabsorption [19], and mediates tumor cytostasis [20, 21].

IL-1 has been shown to provide a signal for T-cell proliferation, in response to both antigen-specific and polyclonal T-cell stimulation [22, 23], but it is not required for the recognition of an exogenous antigen provided by an Ia identical cell [24–26] and cannot substitute for accessory cells in MHC-restricted antigen presentation.

It has been proposed that IL-1 induces T cells to produce mitogenic lymphokines such as interleukin-2 (IL-2) and to express their respective receptors on T cells [27–31]. The synergistic action of IL-1 with IL-2 and IFN in boosting NK activity has also been reported [32]. Furthermore, an in vitro role of IL-1 in enhancing the proliferative response of activated B cells to B-cell growth factors [33–36] and in modulating antibody production [37, 38] has been suggested.

In an immune response, IL-1 is released by macrophages after activation by two pathways: one is the genetically unrestricted direct challenge of macrophages by various compounds including lipopolysaccharides, purified tuberculin protein derivatives, muramyl dipeptide, phorbol myristate acetate, silica particles, and the glucocerebroside GL-1 [6]. A second pathway for the secretion of IL-1 involves activated T cells that stimulate monocytes to produce IL-1 by a cell contact-dependent, genetically

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* This work was supported by grant He 1380-2/1 from the Deutsche Forschungsgemeinschaft and grant 0319015 from the Bundesministerium für Forschung und Technologie

restricted pathway which requires Ia antigen identity [39, 40]. Recently, ligand binding to monocyte membrane structures as provided by monoclonal antibodies to Ia antigens has been reported to stimulate monocyte IL-1 secretion [41]. Also, lymphokines released by T cells, such as the granulocyte/monocyte colony-stimulating factor, have been implicated in monocyte IL-1 secretion [42].

However, little is known about mechanisms underliying T-cell/monocyte interactions that lead to monocyte IL-1 secretion.

We have previously shown that T-cell-derived γ-IFN induced binding sites for IL-2 on monoblast line U 937 and the promyelocyte line HL 60 that bound biologically active IL-2 [43]. In addition, we have presented evidence for IL-2-R expression on γ-IFN treated human peripheral blood monocytes [43]. We have now shown, by means of biochemical and molecular identification and bioassays, that cultured human peripheral blood monocytes display binding sites for IL-2. Stimulation of monocytes with γ-IFN, lipopolysaccharide (LPS), or phytohemagglutinin (PHA) enhanced surface IL-2-R expression up to threetimes. Binding of IL-2 to monocyte IL-2-R resulted in IL-1 secretion that could be enhanced five- to sixfold when monocytes were costimulated with LPS. Moreover, the progressive loss of monocyte IL-1 secretion upon monocyte aging in vitro was reversed by either IL-2 or γ-IFN alone, but was most effective in the presence of both factors. IL-2 effects on IL-1 secretion by highly purified monocyte preparations could be partially blocked by the addition of anti-IL-2-R antibodies to the cultures.

These results provide insights into the T-cell/monocyte interactions that lead to monocyte IL-1 secretion, suggesting that the T-cell lymphokines IL-2 and γ-IFN may act on monocytes to amplify the immune response by establishing a positive feedback loop.

B. Materials and Methods

I. Separation of Monocytes

Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteer donor buffy coats by Ficoll-Hypaque (density 1.077 g/cm) gradient centrifugation. T cells were recovered by rosetting with AET-treated SRBC (5% vol/vol solution); monocytes were separated by repeated adherence steps of the E-rosette negative fraction [44]. Purity was determined by Wright-Giemsa staining, ANAE staining, and immunofluorescence analysis with monoclonal antibodies to the Mo-2, T-11, B-1, and NKH1-A antigens [45–48].

II. Culture Conditions

In some experiments monocytes were incubated at 1×10^6 cells/ml in 60×15 mm Petri dishes (Falcon, Oxnard, CA) at 37° C in a 5% CO_2 atmosphere and cultured for a period of 12–72 h in the presence or absence of 50–1000 U/ml (SA = 6×10^7 U/mg) of purified *Escherichia coli*-derived recombinant γ-IFN (Biogen Research Corporation, Cambridge, MA). The culture medium was RPMI 1640 supplemented with 5% low-endotoxin FCS, 2 m*M* L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% sodium pyruvate (Gibco, Grand Island, NY) (= standard culture medium).

In selected experiments other compounds potentially affecting monocyte functions were added, either alone or in combination, for 48 h to monocyte cultures, including prostaglandin E_2 (10^{-6} *M*), LPS; *E. coli* 026: B6 (15 µg/ml, Sigma, St. Louis, MO), PHA (2 µg/ml, Burroughs-Wellcome, Greenville, NC), 1.25 (OH) vitamin D_3 (10^{-8} *M*), recombinant αA-IFN, β-IFN (500 U/ml; SA = 2×10^8 U/mg, Hoffman-La Roche, Nutley, NJ) and recombinant TNFα (150 U/ml; SA = 7.2×10^7 U/mg, Genentech, San Francisco, CA). Cells derived from cultures as described above

were subjected to immunofluorescence staining and analysis.

In other experiments designed to examine the effects of γ-IFN and IL-2 on modulation of IL-1 secretion by monocytes, monocytes were cultured in 24-well, 16-mm flat-bottom plates (Falcon) at 2.5×10^5 cells/ml and 1 ml/well for 24–168 h in the presence or absence of γ-IFN (250 U/ml), recombinant IL-2 (2.5–500 U/ml; SA = 10^6 U/mg, Biogen) or a combination of both lymphokines, IL-1-inducer LPS (15 μg/ml), anti-IL-2-R 1 mcAb [49] (diluted 1:100–1000), or control IgG2a mcAbs, that were either binding or nonbinding to monocytes (diluted 1:100). Supernatants were harvested after various culture times and subjected to IL-1 and IL-2 bioassays.

In some experiments (indicated in the table headings) supernatants were treated with 1500 nU/ml of a neutralizing mcAb to γ-IFN, 3C11C8 [44], and mcAb to IL-2 (DMSl, 0.5 mg/ml; kindly provided by Dr. K. A. Smith) for 2 h at 27° C. In other selected experiments supernatants were additionally absorbed with 1×10^7 cloned murine IL-2-dependent CTLL cells at 4° C for 24 h to remove IL-2 activity that was potentially not neutralized. Culture medium in this culture type was standard culture medium supplemented with indomethacin (10^{-6} *M*, Sigma) to prevent the endogenous secretion of prostaglandins, known to inhibit IL-1 release [50].

III. Immunofluorescence Staining

For one-color staining, 1×10^6 fresh or cultured monocytes were incubated for 30 min at 4° C with the appropriate mcAbs to monocyte-, T-cell-, B-cell-, NK-cell antigens (Mo-2, T-11, B-1, NKH1-A) or several antibodies to IL-2-R, including anti-IL-2-R 1 [49]), anti-TAC [51], and anti-7G7/B 6 [52] (kindly provided by Drs. T. Waldmann and D. Nelson) or irrelevant isotype-identical control antibodies. If antibodies were not directly fluoresceinated, a second incubation was performed for 30 min at 4° C with fluorescein-conjugated goat anti-mouse IgG + IgM (Tago, Burlingame, CA). The washing medium was minimal essential medium (MEM; Gibco), containing 10% pooled human AB serum. For dual fluorescence studies, monocytes that had been treated with γ-IFN, PHA, or LPS were incubated with biotin-conjugated anti-Mo-2 and a fluoresceinated anti-IL-2-R 1. After washing, cells were developed with avidin-conjugated Texas red (Molecular Probes, Junction City, OR). Cells were analyzed using a dual-laser flow cytometer (EPICS V; Coulter Electronics, Hialeah, FL).

IV. Assay for IL-1

Single thymocyte suspensions from C3H/HeJ mice (female, 6–8 weeks old) (Jackson Laboratory, Bar Harbor, ME) were cultured at 1.5×10^6 cells/150 μg/well in standard culture medium supplemented with 2.5×10^{-5} *M* 2 mercaptoethanol (2 ME; Sigma) and submitogenic concentrations of PHA (0.5 μg/ml) in the presence or absence of supernatant conditioned by monocytes (25% vol/vol) for 72 h at 37° C, 5% CO in 98-microwell flat-bottom plates (Falcon) as described [53]. Eighteen hours before harvesting, cultures were pulsed with 1 mCi/ml = 37 kBq/ml of tritiated-thymidine (^{3}H-Td; Schwartz-Mann, Spring Valley, NY). The incorporated radioactivity was collected onto fiberglass filters and assayed using a liquid scintillation counter (Packard Instruments, Downer's Grove, IL). The levels of IL-1 activity in supernatants tested are expressed as cpm values of ^{3}H-Td incorporated by thymocytes (mean of triplicate cultures). In selected samples IL-1 activity present in the supernatants was detected using the LBRM 33-IA5B6 conversion assay [54].

V. Assay for IL-2

Interleukin-2 activity was determined by assaying the growth of IL-2 dependent murine CTLL 2 cells as described [55]. CTLL 2 cells were cultured at 5×10^6

cells/100 µl/well at 37° C, 5% CO_2, in 98-microwell flat bottom plates (Falcon) in standard culture medium supplemented with 5×10^{-5} *M* 2 ME in the presence or absence of monocyte conditioned supernatants (25% vol/vol) for 48 h. The cells were pulsed with 3 H-Td for the final 18 h before harvesting. The incorporated radioactivity was measured in the same manner as described for the IL-1 assay. IL-2 levels are expressed as cpm values of 3 H-Td incorporated by CTLL 2 cells in response to IL-2 (mean of triplicate cultures).

VI. Iodination, Immunoprecipitation, and SDS-PAGE

Monocytes treated for 48 h with 250 U/ml γ-IFN were externally labeled with 125 I using the lactoperoxidase-catalyzed method. Immunoprecipitates with anti-IL-2-R 1 antibody were analyzed by sodium dodecyl–10% polyacrylamide gel electrophoresis (SDS-PAGE) [43].

VII. Northern Blot Analysis

Total cellular RNA was prepared employing the guanidinium isothiocyanate/cesium chloride method [23]. Monocytes were lysed in situ, adherent on the Petri dishes. After glyoxylation, 10-µg samples of RNA were size-fractioned by agarose gel electrophoresis and transferred onto synthetic membranes. Filters were hybridized with a full-length IL-2-R cDNA (kindly provided by Dr. W. C. Greene), radiolabeled, and exposed to Kodak XAR5 using Dupont Cronex intensifying screens. To exclude contamination of monocyte RNA with RNA derived from T cells possibly contaminating monocyte culture, filters were reprobed with a cDNA specific for the T-cell receptor β-chain gene (kindly provided by Dr. H. D. Royer).

C. Results

I. Purification of Monocyte Preparation

Isolation of monocytes by two sequential adherence steps of E-rosette-negative PBMC resulted in cell preparations consisting of >98% monocytes by morphology and cytochemistry (Wright Giemsa and ANAE staining). Cytofluorographic analysis of these cells employing mcABs to Mo-2 (monocytes), T-11 (T cells), B-1 (B cells), and NKH1-A (NK cells) revealed 96%–99% Mo-2-positive cells. Reactivity with anti-T-11, -B-1, and -NKH1-A was below the background fluorescence. The possibility that the few remaining T cells, present in the monocyte cultures and not detectable by means of morphology or immunofluorescence, produced endogenous IL-2 was examined by assaying samples of IL-1-containing conditioned medium for their effects on IL-2-dependent CTLL 2. A rat IL-2 sample used for maintenance of CTLL 2 cells served as a positive control. None of the conditioned media tested exhibited any activity on the CTLL 2 cells (data not shown).

II. Constitutive and Modulated IL-2-R Expression on Peripheral Blood Monocytes

IL-2-R expression is negligible in freshly isolated monocytes (<2%). However, using mcAb anti-IL-2-R 1 and immunofluorescence, surface IL-2-R were detectable in monocytes after 24 h of culture, with a maximum (25%) after 48–60 h. Exposure of monocytes to r γ-IFN (50–1000 U/ml) resulted in enhanced expression of binding sites for anti-IL-2-R 1; they were at a maximum when 100 U/ml r γ-IFN was present during 60 h of culture (Table 1). Similar results were obtained using other anti-IL-2-R antibodies, such as anti-TAC and anti-7G7/B6 (data not shown).

Identification of these binding sites as IL-2-R was confirmed by immunoprecipitation of the same 60- to 65-kD protein

Cultures of monocytes	IL-2-R1 Number of stained cells (%)
Nontreated	25[a]
Treated with	
γ-IFN	63
αA-IFN	25
β-IFN	24
TNFα	24
1.25 (OH) vitamin D_3	26
PGE2	23
LPS	52
PHA	55
γ-IFN + TNFα	74
γ-IFN + PGE2	39
γ-IFN + LPS	64
γ-IFN + αA-IFN	64
γ-IFN + β-IFN	60

Table 1. Effect of various compounds (48-h exposure) on IL-2-receptor expression of cultured peripheral blood monocytes

See text for concentrations used. Recovery of viable cells in each fraction was >78%.

[a] Data of one representative experiment.

from surface-labeled PHA-activated T cells (72 h, 2 μg/ml) as from γ-IFN-induced cultured peripheral blood monocytes (60 h, 100 U/ml; Fig. 1). Other species of IFN, such as r α-A-IFN or r β-IFN, or compounds that are known to be involved in the modulation of monocyte/macrophage functions, such as PGE_2, 1.25 (OH) 2 vitamin D_3, and TNF α, failed to enhance monocyte surface IL-2-R expression. However, when various combinations of these compounds were assayed, it was demonstrated that PGE_2 partially inhibited the γ-IFN-induced enhancement of IL-2-R on monocytes, whereas TNF α – although it demonstrated no enhancing effect on IL-2-R expression by itself – synergized with γ-IFN to increase the number and fluorescent intensity of IL-2-R 1 + monocytes (data not shown), while cocultures of γ-IFN together with αA-IFN, β-IFN, and LPS did not alter the effects observed with γ-IFN alone (Table 1).

PHA and LPS also increased the number of IL-2-R 1 + monocytes. This was confirmed by two-color immunofluorescence using biotin-conjugated anti-Mo-2 and fluoresceinated anti-IL-2-R 1 antibody (Fig. 2). Of the cells used for two-color immunofluorescence studies, 97.2%–99.4%) were stained by biotinylated Mo-2; 53.5%, 59.2%, and 62.5%, respectively, of cells from PHA-, LPS-, and γ-IFN-treated cultures were double-stained by biotinylated Mo-2 and fluoresceinated anti-IL-2-R 1. To determine whether monocyte surface expression of IL-2-R was associated with induced expression of the IL-2-R gene, cytoplasmic mRNA of monocytes treated with LPS, PHA, or γ-IFN was extracted and hybridized to an IL-2-R gene-specific cDNA. As shown in Fig. 3, unstimulated monocytes failed to accumulate transcripts for IL-2-R. In the presence of the stimulatory compounds, however, the IL-2-R message of 3.5 and 1.5 kb in size, became detectable 12 h after initiation of cultures. These mRNA were similar in size to the corresponding messengers seen in PMA/PHA-activated normal T cells. Lack of T-cell receptor-β-chain transcripts in RNA derived from monocyte cultures excluded the possibility that T-cell contamination was responsible for the IL-2-R messenger (data not shown).

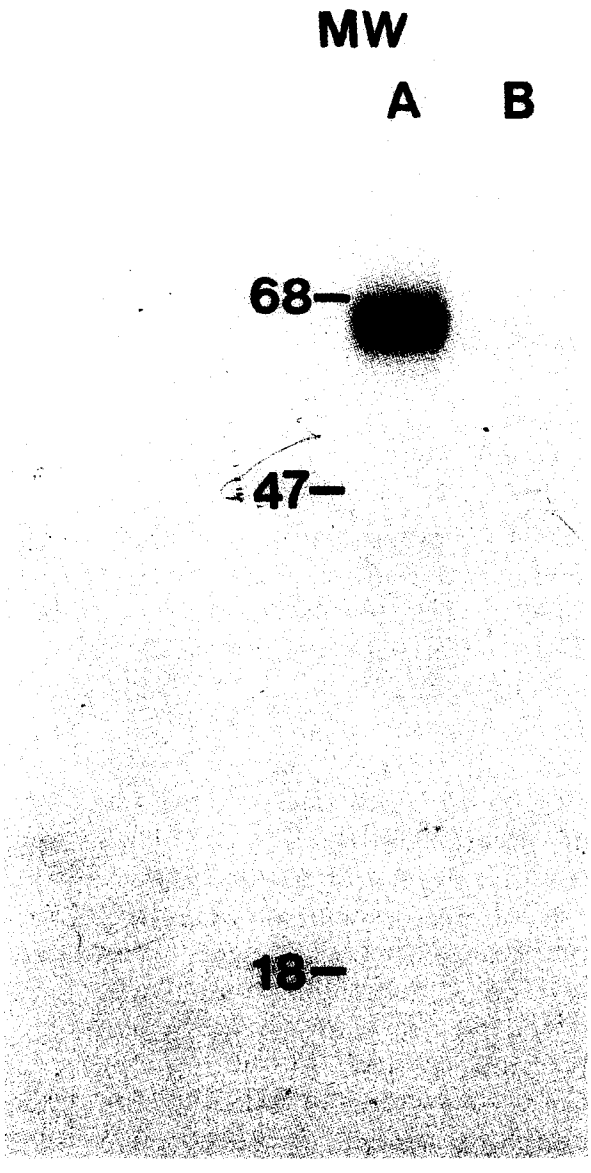

Fig. 1. SDS-PAGE analysis of immunoprecipitates obtained with anti IL-2-R 1 antibody from γ-IFN-treated (48 h, 250 U/ml) monocytes (*lane A*) under reducing conditions. *Lane B* represents control immunoprecipitates from the same cells using an unreactive control antibody (anti-glycophorin A)

III. γ-IFN and IL-2 Enhance LPS-induced and Maintain LPS-inducible IL-1 Release by Cultured Peripheral Blood Monocytes

When monocytes were cultured for 24 h, subsequent stimulation with LPS (15 μg/ml) resulted in the release of IL-2 activity in the supernatants. Costimulation with 250 U/ml r γ-IFN at initiation of the cultures yielded a twofold increase in the level of IL-1 activity when monocytes were induced with LPS. As seen in Table 2, when r IL-2 (500 U/ml) was present during the first 24 h of culture and was thoroughly washed off before inducing with LPS, an up to 30% increase in detectable IL-1 activity was observed, as compared with untreated LPS-induced cultures.

However, IL-2 synergized with γ-IFN to yield a sixfold enhancement of LPS-inducible secretion of IL-1 activity, whereas LPS-uninduced cells did not secrete significant levels of IL-1. None of the supernatants assayed for IL-1 activity showed any mitogenic stimulation of IL-2-dependent CTLL 2 cells (data not shown).

Monocytes cultured for more than 84 h were diminished in their ability to support LPS-inducible IL-1 production. This loss of IL-1 secretory potential is complete after 214 h, but it can be overcome by the addition of IL-2 or γ-IFN, and more dramatically by a combination of both lymphokines (Table 3) when introduced at the time of initiation of cultures. When cultures were performed in the presence of IL-2, γ-IFN, or both, the ability of LPS to induce the release of IL-1 was largely maintained and the mitogenic activity in supernatants remained comparable to that in fresh cultures. Again, when cultures were washed thoroughly before LPS was introduced no IL-2 activity could be detected in the supernatants to be assayed for IL-1 activity (not shown).

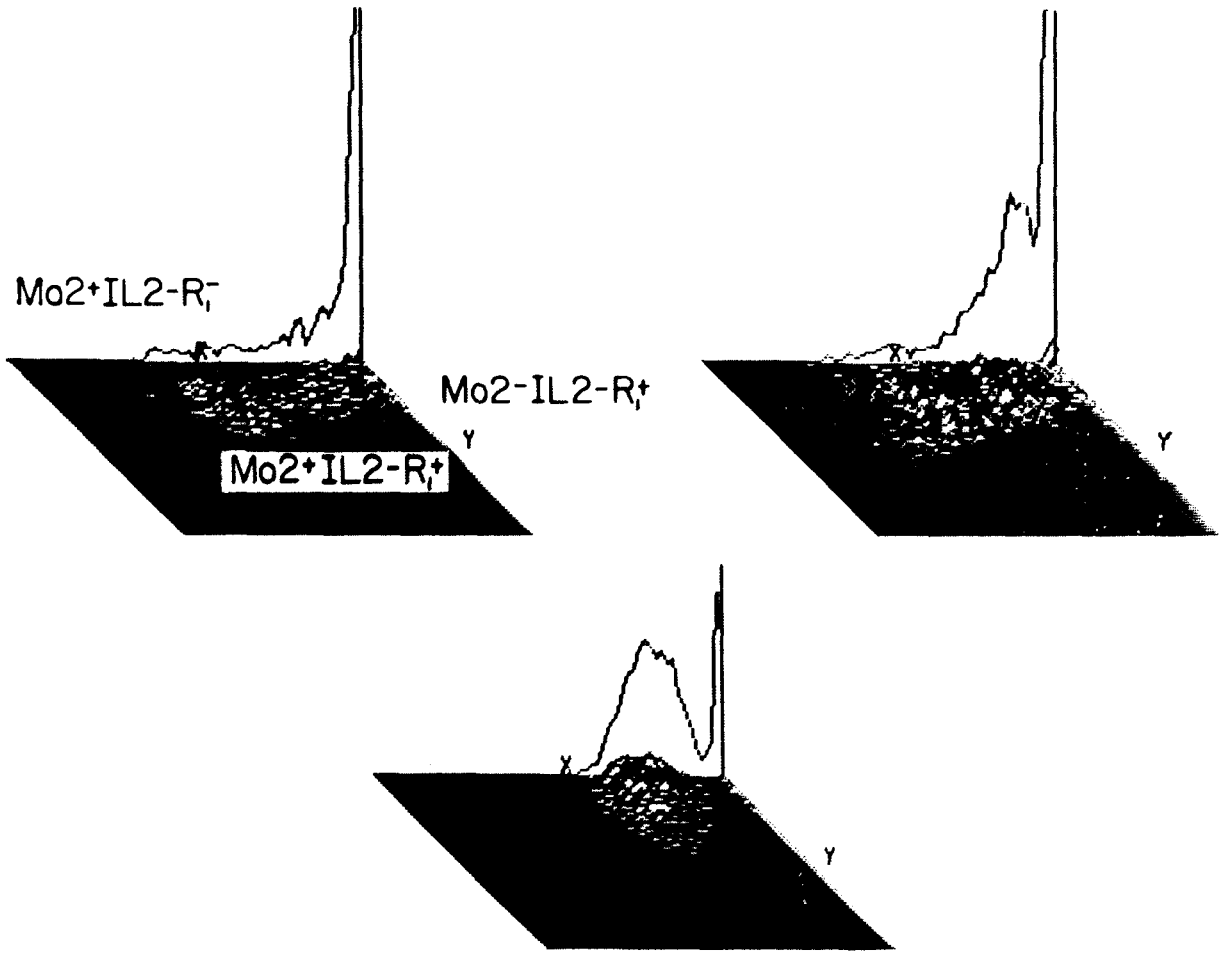

Fig. 2. Dual-color fluorescence analysis of IL-2-R expression on peripheral blood monocytes cultured for 48 h in the presence of LPS (15 μg/ml; *top left panel*), PHA (2 μg/ml; *top right panel*), or γ-IFN (250 U/ml; *bottom panel*). Mo-2 antigen was detected with biotin-conjugated anti-Mo-2 (*x axis*), and IL-2-R was detected with fluoresceinated IL-2-R 1 antibody (*y* axis)

Table 2. γ-Interferon and interleukin-2 enhancement of LPS-induced interleukin-1 release by cultured monocytes

Culture of monocytes in:	^{3}H-Td Incorporation[a] of murine thymocytes induced by monocyte-conditioned media[b] (MCM) stimulated with:					
	Culture medium			LPS		
	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3
Culture medium	368	127	309	6687	9859	5392
γ-IFN	446	141	287	12036	18329	11007
IL-2	439	213	308	7914	9997	6985
γ-IFN + IL-2	471	242	357	36875	33341	29890

Monocytes (2.5×10^5/ml) were cultured in culture medium in the absence or presence of γ-IFN (250 U/ml), IL-2 (500 U/ml), or a combination of both for 24 h. After several washings, cultures were reincubated in fresh culture medium with or without LPS (15 μg/ml).
After further 24 h, cell-free supernatants were harvested and assayed for IL-1 biological activity.
[a] Values are expressed as cpm of triplicate cultures. SD never exceeded 7%. ^{3}H-Td Incorporation of thymocytes stimulated with PHA (0.5 μg/ml) or PHA + LPS (15 μg/ml) was 264 ± 67 and 339 ± 71.
[b] The result given correspond to a dilution of MCM of 25% vol./vol.

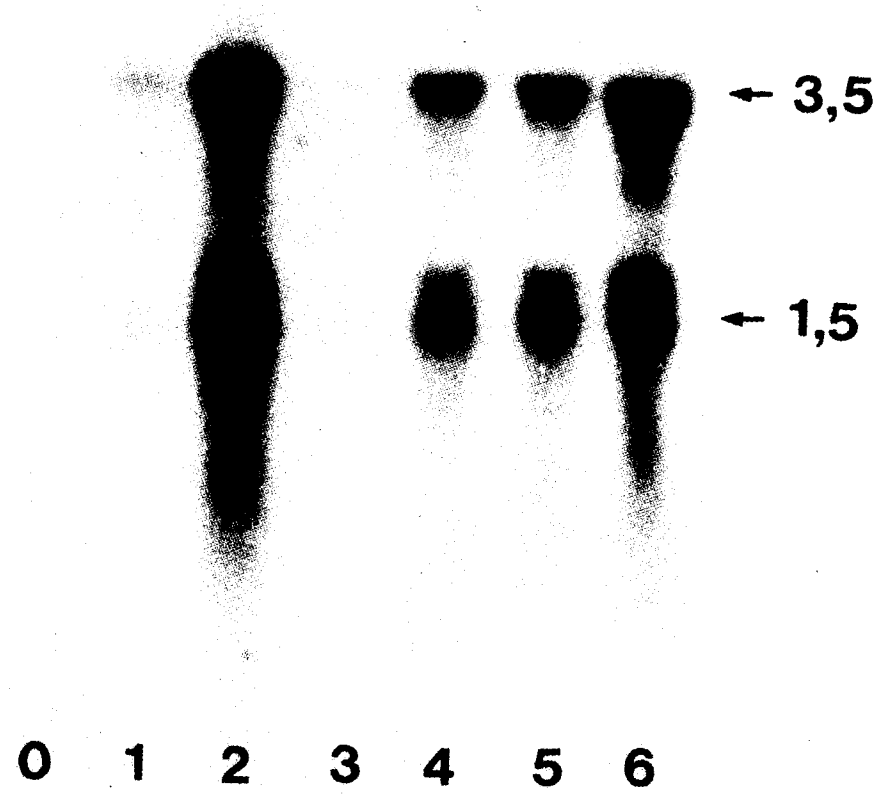

Fig. 3. Detection of IL-2-R mRNA in monocytes cultured for 12 h in the presence of LPS (15 μg/ml; *lane 4*), PHA (2 μg/ml; *lane 5*), or γ-IFN (250 U/ml; *lane 6*). Monocytes cultured in the presence of medium alone (*lane 3*) failed to display IL-2-R mRNA. Lanes 0, 1, and 2 represent control lanes of T cells cultured for 12 h in medium alone (*lane 0*), in the presence of PHA (2 μg/ml; *lane 1*), or PHA plus PMA (10^{-9} *M*; *lane 2*)

IV. Effect of γ-IFN and IL-2 on the Induction of IL-1 Release by Cultured Monocytes in the Absence of LPS

In an attempt to examine whether cultured monocytes were able to release IL-1 activity in response to a cascade of lymphokines, independently from induction by LPS, experiments were performed in which γ-IFN- or IL-2-treated or untreated control cultures (0–144 h) received a second lymphokine pulse, either with γ-IFN or with IL-2. As seen in Tables 4 and 5, when monocytes were cultured in the presence of γ-IFN (250 U/ml), they could be induced to release IL-1 activity by IL-2; this was maximally detectable when γ-IFN was present in the primary culture for 60 h, requiring a minimal IL-2 concentration of ≥250 U/ml (Table 5). When primary cultures were performed in the absence of γ-IFN and with or without IL-2, γ-IFN was unable to induce any IL-1 release (Table 4). Supernatants from secondary cultures, induced with either γ-IFN or IL-2, were treated with neutralizing concentrations of mcAb to γ-IFN (3C11C8) or to IL-2 (DMS1), to avoid a carry-over of antiproliferative effects (although not detectable in control experiments) of γ-IFN or mitogenic activities of IL-2 into the final IL-1 assay. In selected experiments supernatants were additionally absorbed with 1×10^7 IL-2-dependent cloned CTLL cells (24 h, 4° C), to remove any possible residual exogenous IL-2 activity (although not detectable in the CTLL 2 proliferation assay). A possible mechanism by which IL-2 could induce IL-1

secretion, particularly in γ-IFN-primed cultures, would be that γ-IFN enhances the monocyte ability to respond to the presence of IL-2. Therefore, further experiments were designed to examine whether IL-1-enhancing effects of IL-2 were mediated via γ-IFN-induced receptors for IL-2.

V. Effect of Monoclonal Antibody to IL-2-R (anti-IL-2-R 1) on IL-1 Release from γ-IFN-pretreated and IL-2-induced Cultures of Peripheral Blood Monocytes

Since γ-IFN was shown to increase threefold the number of monocytes expressing binding sites for anti-IL-2-R 1 antibody, possibly resulting in an enhancement of the ability of monocytes to respond to IL-2, we investigated the effect of anti-IL-2-R 1 antibody on the IL-2-induced IL-1 release from γ-IFN-primed monocyte cultures. Under optimal experimental conditions for release of IL-1 activity in IL-2 (500 U/ml)-stimulated cultures of γ-IFN (250 U/ml)-pretreated monocytes (60 h), the presence of anti-IL-2-R 1 antibody (diluted 1:100) during the last 24 h resulted in a reduction of detectable IL-1 activity in the supernatants by 60%. A 20% reduction of released IL-1 activity was observed when IL-2-induced supernatants were tested that had been generated in the absence of γ-IFN (Table 6), suggesting that IL-1 induction by IL-2 might be mediated at least in part via IL-2-R on cultured monocytes, either induced by γ-IFN or (to a much lower extent) constitutively expressed. However, cultures that were performed in the absence of IL-2, or those in which anti-IL-2-R 1 antibody was replaced by isotype-identical binding and nonbinding control mcAbs were free of detectable IL-1 activity or exhibited unchanged levels of IL-1 release, respectively.

D. Discussion

The interaction of amplifying soluble messenger molecules may be the prereq-

Table 3. γ-Interferon and interleukin-2 maintenance of LPS-induced interleukin-1 release by cultured monocytes

Monocytes in culture (h)	^{3}H-Td Incorporation of murine thymocytes induced by monocyte-conditioned media stimulated with											
	Culture medium			γ-IFN			IL-2			γ-IFN + IL-2		
	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3
48	6384	8173	8064	12987	13112	16507	7801	9231	9201	27340	34107	32998
108	2911	3702	3322	13017	13004	15743	8016	9746	9384	39108	41693	43821
192	897	1965	1713	13223	13897	15789	8092	9787	9402	29974	33342	34502
240	312	ND[a]	ND	11314	ND	ND	7314	ND	ND	21117	ND	ND

Monocytes (2.5×10^5/ml) were cultured in culture medium in the absence or presence of γ-IFN (250 U/ml), IL-2 (500 U/ml) or a combination of both for 24, 84, 168, and 216 h. After several washings, cultures were reincubated with fresh culture medium supplemented with LPS (15 μg/ml) for an additional 24 h and cell-free supernatants were harvested and assayed for IL-1 biological activity. For further details see legend to Table 2.

[a] Not done.

Table 4. Effect of γ-interferon and interleukin-2 on the induction of interleukin-1-release by cultured monocytes in the absence of LPS

Induction culture with γ-IFN	^{3}H-Td Incorporation of murine thymocytes induced by monocyte conditioned media stimulated with								
Monocytes in culture (h)	Culture medium			Culture medium + γ-IFN			Culture medium + IL-2		
	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3
24	293	366	298	246	353	342	258	359	317
84	316	357	353	270	377	359	278	384	339
168	208	199	ND[a]	188	301	ND	243	292	ND
Induction culture with IL-2	^{3}H-Td Incorporation of murine thymocytes induced by monocyte conditioned media stimulated with								
Monocytes in culture (h)	Culture medium			Culture medium + IL-2			Culture medium + γ-IFN		
	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3	Expm. 1	Expm. 2	Expm. 3
24	441	429	407	473	427	421	791	886	809
84	609	594	706	701	600	733	19384	18247	17003
168	489	383	ND	493	394	ND	5604	4041	ND

Monocytes (2.5×10^5/ml) were cultured for various time in culture medium in the absence or presence of γ-IFN (250 U/ml) or IL-2 (500 U/ml). At time point 0, after 60 h and 144 h, cultures that were washed several times received fresh culture medium supplemented with either γ-IFN (250 U/ml) or IL-2 (500 U/ml). After a further incubation period of 24 h, cell-free supernatants were harvested, treated with neutralizing concentrations of monoclonal antibody to γ-IFN (3C11C8; 1500 nU/ml) and monoclonal antibody to IL-2 (DMS 1; 0.5 mg/ml) (2 h at 27 °C) and assayed for IL-12 biological activity. For further details see legend to Table 2.

[a] Not done.

Table 5. Effect of graded concentrations of IL-2 on IL-1 secretion from γ-IFN-treated monocytes

IL-2 (U/ml)	^{3}H-Td Incorporation of murine thymocytes induced by monocyte conditioned medium		
	Expm. 1	Expm. 2	Expm. 3
0	256	293	301
2.5	249	289	317
25	436	301	314
250	5332	4736	6004
500	15937	13889	16019

Monocytes (2.5×10^5/ml) were cultured in the presence of γ-IFN (250 U/ml). After 60 h, graded concentrations of IL-2 (2.5–500 U/ml) were added to the cultures. After further 24 h, cell-free supernatants were harvested, treated with antibodies 3C11C8 and DMS1 (as described in Table 4), absorbed with CTLL cells 24 h, 4 °C), and assayed for IL-1 biological activity. For further details see legend to Table 2.

uisite for the events leading to effector functions of cells participating in an immune response [6]. Production of γ-IFN is closely associated with the production of IL-2 in IL-2-R-bearing T cells, which is regulated at the transcriptional level [56–60]. IL-2-R expression in T cells and IL-2 production is amplified by IL-1 and requires the presence of appropriate MHC products on accessory cells [31] that are in turn regulated by γ-IFN [43, 61–63]. More recently, the T-cell lymphokine TNF-β and monokine TNF-α have been implicated in this cascade: it was demonstrated that both molecules are involved in the cytotoxic effector function of the respective cell type when induced with γ-IFN or IL-2 and when acting synergistically with γ-IFN [60, 64, 65]. Therefore, the study of the regulation of the functional interrelationship of these lymphocyte/monocyte-derived activities may represent an interesting model for the investigation of intercellular communications in the immune response.

Whereas the association of the interactions of the humoral regulator molecules during the intial steps of the cascade of an immune response – i.e., the IL-1-mediated enhancement of IL-2 production, leading to IL-2-mediated enhancement of γ-IFN production, leading to secretion of TNF-α and -β, and the final downregulation of macrophage Ia-expression, IL-1 secretion, and T-cell IL-2 and γ-IFN release by prostaglandins or other cAMP agonists – is well documented [50, 66–68], relatively little is known about mechanisms that contribute to the preservation and perpetuation of these activities.

The studies in this article present evidence for the surface expression of IL-2-R in cultured monocytes and detail their possible implication in the secretion of IL-1. IL-2-R were detected by several anti-IL-2-R mcAbs and were indistinguishable from T-lymphocyte IL-2-R by immunoprecipitation. The prerequisite for monocyte IL-2-R expression was contact with plastic surfaces during culture, since monocyte culture in hydrophobic dishes did not result in IL-2-R expression (data not shown). The time course of monocyte IL-2-R expression that was first detectable after 24 h of culture suggested de novo synthesis rather than the unmasking of cryptic receptors. A several-fold increase in the number of IL-2-R-bearing monocytes was obtained when monocyte cultures were performed in the presence of γ-IFN (100–250 U/ml) or, to a lower extent, in the presence of LPS (15 μg/ml) and PHA (2 μg/ml). This effect was not seen when monocytes were cultured in the presence of purified recombinant preparations of αA-IFN, β-IFN, TNF-α, or other modulators of monocyte function such as PGE_2, and 1.25 (OH) 2 vitamin D_3. However, culture combinations of γ-IFN with TNF-α or PGE_2 lead to a further increase (TNF-α) or decrease (PGE_2) in the number and fluorescence intensity of IL-2-R-expressing monocytes as well as in the levels of surface HLA-DR expression (data not shown), confirming previous observations of synergistic or antagonistic actions of both

Table 6. Effect of monoclonal antibody to interleukin-2-receptor (IL-2-R1) on interleukin-1 by γ-IFN-pretreated monocytes cultured in media containing interleukin-2

Induction culture in	^{3}H-Td Incorporation of murine thymodytes induced by monocyte conditioned media stimulated with							
	Control medium γ-IFN		Control medium + γ-IFN		Control medium IL-2		Control medium + γ-IFN	
	Expm. 1	Expm. 2	Expm. 1	Expm. 2	Expm. 1	Expm. 2	Expm. 1	Expm. 2
Culture medium	282	279	280	286	1433	1291	17355	15137
IL-2-R1 (1:1000)	291	268	301	289	1352	1284	14997	13989
IL-2-R1 (1:100)	286	272	284	289	997	1017	8569	6146
Control antibody 1	274	273	270	278	1439	1276	17158	15206
Control antibody 2	259	268	267	271	1418	1272	17204	15217

Monocytes (2.5×10^5/ml) were cultured in culture medium in the absence or presence of γ-IFN (250 U/ml). After 26 h, cultures received a second γ-IFN (250 U/ml) or an IL-2 (500 U/ml) pulse. After an additional 24 h, monoclonal antibody IL-2-R1 (1:1000 and 1:100 final dilution) or isotype-.identical control binding (1) or nonbinding (2) antibodies were added. After further 12 h of culture, cell-free supernatants were harvested, treated with antibodies 3C11C8 and DMS1 (as described in Table 4), and assayed for IL-1 biological activity. In Expm. 2 supernatants were additionally absorbed with cloned murine CTLL cells (24 h, 4 °C) before assay for IL-1 activity. γ-IFN and antibody to γ-IFN (3C11C8), IL-2 (DMS1), IL-2-R (anti IL-2-R1), and control antibodies had no effect on the thymocyte assay for IL-1 by themselves, nor did they modify the activity of a standard IL-1 preparation (purified IL-1, Genzyme, Norwalk, CT) (not shown). For further details see legend to Table 2.

components with γ-IFN in terms of other macrophage functions [50, 64, 69, 70]. IL-2-R expression in nonlymphoid cells has now been repeatedly documented in transformed cells including monoblast line U 937 [43] and promyelocytic line HL 60, when differentiation along the monocytic axis was induced with γ-IFN [43, 71], whereas myeloblast line KG 1 [43] and a newly established line derived from a human eosinophilic leukemia expressed IL-2-R [72] in the absence of exogenous inducing agents. Although we were unable to detect inducible or constitutively expressed IL-2-R on mouse peritoneal macrophages using antimouse IL-2-R antibody AMT 13 (T. Diamantstein and F. Herrmann, unpublished observations), in line with previous reports that functions of macrophages derived from different sites or sources may be regulated differentially [73–75], the recent finding of low-affinity IL-2-R on murine IL-3-dependent myelomonocytic cell lines [76] demonstrates the lack of possible interspecies differences.

To examine the biological consequences of IL-2-R expression by human peripheral blood-derived monocytes, in particular when upregulated by γ-IFN, we assessed the effects of the interactive cascade of both lymphokines on the regulation of monocyte IL-1 secretion, since it has been shown that T-cell products may induce IL-1 secretion in the absence of cell-to-cell contact [39, 40, 53]. Every attempt was made, and a large array of control experiments were introduced to rule out any contaminants that would cause mitogenic activity other than that of IL-1, including the use of low-endotoxin serum, highly purified preparations of recombinant lymphokines, and highly enriched monocyte preparations. In addition, we excluded the presence of exogenous or endogenously generated biologically active lymphokines in the final IL-1 assay, using neutralizing mcAbs to γ-IFN and IL-2 as well as absorption techniques to purify the supernatants to be assayed for IL-1 from IL-2 activity by using an IL-2-dependent murine T-cell clone.

Under these experimental conditions our results can be summarized as follows: Monocytes do not secrete IL-1 in the absence of inducers such as LPS, IL-2, or γ-IFN, and, more effectively, the synergistic action of both lymphokines results in an up to sixfold enhancement of LPS-inducible IL-1 secretion. The loss of monocytes to release IL-1 due to aging in culture was overcome when monocytes were cultured in the presence of γ-IFN, IL-2, and, more effectively, in the presence of *both* T-cell products before induction with LPS. These results confirm previous observations by other groups in some aspects [26, 63, 77]. Whereas γ-IFN seems not to have any IL-1-inducing property, an IL-1-inducing capacity was demonstrated for IL-2, although concentrations of more than 250 U/ml were necessary.

The capacity of IL-2 to induce IL-1 secretion required at least in part the presence of IL-2-R on monocytes, as demonstrated by blocking experiments with anti-IL-2-R mcAbs. Prior enhancement of the number of IL-2-R-bearing monocytes by γ-IFN resulted in an about 30fold increase of IL-2-mediated secretion of IL-1 activity, suggesting a fundamental role for the sequential action of both lymphokines in establishing a positive feedback loop to generate IL-1 secretion, and thus to preserve and perpetuate biological activities required for immune responses.

Current studies are underway to address the role of IL-2 itself in modulating levels of IL-2-R in monocytes. In addition, it has been suggested that subpopulations of cultured monocytes may be unable to secrete IL-1 upon induction [73], in particular, those that are involved in counter-regulatory monocyte functions. Expression of IL-2-R on these cells, which could lead to adsorption of IL-2 activity and thus serve as a signal to limit cytotoxic events at inflammatory sites, needs further investigation.

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