IMMUNOCYTOCHEMICAL DETECTION OF INTERLEUKIN 1
WITHIN STIMULATED HUMAN MONOCYTES

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The term IL-1 has been used to describe a family of monocyte derived
dipeptides that have immunomodulatory and proinflammatory biological prop-
erties (1, 2). While most studies have focused on IL-1 activity that is found in
monocyte/macrophage culture supernatants, early studies showed that high
amounts of activity could also be recovered from cell lysates. These studies
suggested that IL-1 may accumulate within mononuclear phagocytes in a pre-
cursor form before release into the surrounding medium (3-5).

Recently it has been shown that there are at least two distinct human IL-1
molecules termed IL-1α and IL-1β. Separate mRNAs for these IL-1 species each
code for a precursor of ~31 kD, which is subsequently processed by still
undefined mechanisms to a “mature” molecule of ~17.5 kD (6). The absence of
a typical hydrophobic leader sequence in either of the IL-1 molecules identified
to date suggests that they may not be typical secretory proteins. A 33-kD
precursor for murine IL-1, which is ~62% homologous to human IL-1α (6), has
been documented at the protein level in cell lysates of peritoneal macrophages,
as well as in lysates of the murine macrophage line P388D1 (7). Comparable
studies have not yet been reported for human monocytes and macrophages.

The successful development of highly specific heterologous antisera (8) for the
species of human IL-1 having a pI of 6.8 (IL-1β) (6, 9) has allowed us to identify
its precursor in the cytoplasm of activated human monocytes. In the present
report, the intracellular accumulation of IL-1 is documented using indirect
immunofluorescence performed on fixed and permeabilized cells, and immuno-
 blot analysis of cell lysates. These studies show that it is possible to identify IL-1-
producing cells at the light microscopic level and suggest that anti-IL-1 antibodies
may be useful for the in situ localization of IL-1 production in inflamed tissue.

Materials and Methods

Antisera. A detailed description of the peptides used, as well as the methodology for
raising and characterizing antisera, is presented elsewhere (8). Briefly, peptides Ala17-
Asp128 (p117-128) and Gln197-Phe215 (p197-215) of the amino acid sequence derived from
IL-1 cDNA (6, 10) were synthesized using standard solid phase methodology (11), coupled
to KLH with the heterobifunctional reagent m-maleimidobenzyl-N-hydroxysuccinimide

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ester (12), and used to immunize rabbits. p117-128 represents the amino terminus of mature secreted IL-1β (9), while p197-215 is an internal amino acid sequence of the same molecule. Each rabbit received 200 μg of peptide conjugate in CFA at multiple intradermal sites. Booster injections of antigen in IFA were then administered at multiple subcutaneous sites 2 and 5 wk later. Solid-phase RIA with the resulting antisera showed that they bound IL-1β in a dose-dependent manner and had half maximal titers at dilutions between 10^{-5} and 10^{-4}. Limjuco et al. (8) further showed the antisera to be monospecific for IL-1 in immunoblot analyses of crude, concentrated culture supernatants from stimulated human monocytes.

Preimmune serum that was collected from each rabbit before its first injection, was used, as appropriate, in control experiments. In addition a rabbit anti-KLH serum was used (Cappel Laboratories, Cochranville, PA).

**Cell Cultures.** Human mononuclear cells were isolated from the blood of normal volunteers by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) discontinuous density gradients. The cells obtained were suspended in RPMI medium containing 1% FCS (Gibco Laboratories, Grand Island, NY), 20 mM Hepes, 100 U/ml penicillin, and 100 μg/ml streptomycin; they were then seeded into 35-mm culture plates containing circular glass coverslips and incubated at 37°C in 5% CO₂/air for specified periods of time. 2-3 × 10⁶ cells were plated per dish. For experiments on stimulated monocytes and lymphocytes, 1 μg/ml LPS from *Escherichia coli* O55:B5 (Sigma Chemical Co., St. Louis, MO) and 5 μg/ml PHA (Sigma Chemical Co.) were added to the culture medium (9). In some experiments 8 μm latex particles (Dow Diagnostics, Indianapolis, IN) were also added to identify phagocytic cells. The final concentration of latex particles was .005% solids/ml medium. All cultures were washed three times with serum-free medium immediately before fixation. The resulting cultures contained ~70% monocytes and 30% lymphocytes.

**Immunofluorescence.** For immunofluorescent analyses, unless otherwise indicated, cell cultures were fixed for 20-25 min at room temperature in 2% paraformaldehyde as described by McLean and Nakane (13). The cells were then permeabilized by treatment with 0.1% Triton X-100 in PBS for 5 min at 4°C or acetone for 30 min at −20°C, and washed well with PBS. Such preparations, if stored in PBS at 4°C, were stable for several weeks. Before staining, the cultures were briefly treated with Tris-HCl, pH 7.8, and then incubated for 20 min with 0.1 M phosphate buffer (pH 7.8) containing 5% nonfat dry milk, 0.1% BSA, and 0.04% sodium azide to minimize nonspecific antibody binding (I. I. Singer, Merck Research Laboratories, personal communication). After several washes with Tris-HCl, the cultures were incubated with immune (anti-IL-1 or anti-KLH) or preimmune serum, diluted 50-100 times, washed, and stained with a 1:100 dilution of FITC-conjugated goat anti-rabbit IgG (Cappel Laboratories). All antibodies were diluted in 0.1 M phosphate buffer containing 0.1% BSA, 0.04% azide, and 0.1% nonfat dry milk and centrifuged to remove particulates before use. Specimens were viewed on a fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) equipped for epillumination with a narrow band selective filter combination for FITC. Micrographs were prepared using Tri-X film (Eastman Kodak Co., Rochester, NY) developed at 20°C for 18 min with acufine developer (Acufine Inc., Chicago, IL).

**Blocking of Fluorescent Staining with Excess Antigen.** To determine the specificity of the staining reaction, diluted antisera (1:100) was preincubated with: (a) relevant IL-1 peptide (unconjugated; 50 μg/ml), (b) irrelevant IL-1 peptide (unconjugated; 50 μg/ml), or (c) OVA (100 μg/ml) for 2.5 h at room temperature. The serum was then used for indirect immunofluorescent staining as described above.

**SDS-PAGE and Immunoblotting.** For analyses of conditioned medium, culture supernatant was collected and concentrated 100 times by ultrafiltration (9) before addition of an equal volume of Laemmli sample buffer (2X). For analyses of cell lysates, mononuclear cell cultures were washed with serum-free medium, scraped into HBSS containing 5 mM N-ethylmaleimide, 2 mM PMSF, and 2 mM EDTA, pelleted, and then resuspended in Laemmli sample buffer for SDS-PAGE. All samples were boiled for 5 min and electro-
phoresed under reducing conditions on 15% polyacrylamide gels with 3% stacking gels as previously described (14).

For immunoblotting, proteins were electrophoretically transferred to nitrocellulose filters (15). The filters were then treated with 0.5% gelatin in PBS for 1 h before treatment with a 1:100 dilution of preimmune or immune serum, followed by 125I-labeled protein A (2 × 10⁵ counts/ml, sp act = 2–4 μCi/μg). Autoradiographs were prepared using XAR film (Eastman Kodak Co., Rochester, NY). Apparent molecular weights of labeled bands were determined by comparison with standard proteins including phosphorylase B (M, 94,000), BSA (M, 67,000), OVA (M, 43,000), carbonic anhydrase (M, 30,000), soybean trypsin inhibitor (M, 20,000), and α-lactalbumin (M, 14,400).

Results

Fluorescent Staining of Fixed and Permeabilized Mononuclear Cell Cultures. To determine whether IL-1 could be detected within stimulated cells via immunocytochemical techniques, human PBMC were cultured for 4.5 h in the presence of LPS and PHA (to stimulate monocytes as well as lymphocytes) and then fixed and permeabilized as described in Materials and Methods. When such preparations were stained using antiserum against the p117-128 peptide of IL-1β, and an FITC-conjugated goat anti-rabbit IgG, intense cytoplasmic fluorescence was observed in cells that were identified by nuclear morphology as monocytes (Fig. 1, A and B). No staining was seen in lymphocytes or platelets present in the same preparations. Control experiments showed that when preimmune serum (Fig. 1, C and D) or anti-KLH serum (not shown) was substituted for the p117-128 antiserum, no fluorescent staining was obtained. Antiserum to the p197-215 internal peptide of IL-1, however, produced staining that was indistinguishable from that seen with the antiserum to the amino terminal peptide p117-128 (data not shown).

To confirm the identity of the cells stained by our anti-IL-1 antisera, as well as to assess the proportion of phagocytic cells containing detectable amounts of IL-1, experiments were carried out in which 8-μm latex beads were added during the culture period. After 4.5 h in LPS- and PHA-containing medium, the cells were fixed, permeabilized, and stained as before. Once again, cultures treated with preimmune serum showed no fluorescence, while in cultures treated with immune serum there was a clear correspondence between fluorescent staining and ingestion of latex (Fig. 2). Scoring of cells in duplicate cultures (25 random fields per culture, 500 × magnification) showed that 93% of the cells that contained three or more latex beads were also positive for IL-1 as judged by immunofluorescence. 95% of the IL-1⁺ cells contained three or more latex particles. Thus, under the described culture conditions, the vast majority of mononuclear phagocytes contained immunocytochemically detectable IL-1, while those cells that did not phagocytize latex almost always lacked fluorescent staining. The small number of cells that were fluorescent and did not contain ≥3 latex beads, invariably were the same size and had the characteristic nuclear morphology of monocytes. Presumably with higher concentrations of latex or longer incubation periods these cells would have proven to be phagocytic.

In view of the observed fluorescent staining of stimulated monocytes, it was also of interest to examine nonstimulated cells and determine whether they too contained immunocytochemically detectable antigen. Accordingly, mononuclear
FIGURE 1. Indirect immunofluorescent staining of an LPS- and PHA-stimulated human mononuclear cell culture. (A) Staining with antiserum against the p17-128 peptide of IL-1β. (B) Phase contrast micrograph of the same field shown in A. (C) Staining with preimmune serum. (D) Phase contrast micrograph of the same field shown in C. Bar, 50 μm. × 583.
FIGURE 2. Indirect immunofluorescent staining of a mononuclear cell culture that had been incubated with LPS, PHA, and latex beads for 4.5 h. (A) Staining with antiserum against the p17-128 peptide of IL-1β. (B) Phase contrast micrograph of the same field shown in A. Note the intense fluorescent staining of cells that have ingested latex and the lack of staining in lymphocytes (arrowheads). Bar, 30 μm. × 925.

cells were isolated as before and suspended in LPS- and PHA-containing culture medium. A portion of the cell suspension was then immediately placed on ice and a cytofuge was used to pellet the cells onto slides. The remaining cells were allowed to adhere to glass coverslips and incubated at 37°C (in the presence of LPS and PHA) for 4.5 h. When such preparations were fixed, permeabilized, and treated with immune serum followed by FITC-conjugated second antibody, the stimulated monocytes, as expected, showed intense fluorescence, while cells that had been suspended in the same medium but then immediately cytofuged and fixed showed no fluorescence. Additional experiments confirmed these results (see below) and showed that cells fixed after only short periods of time in culture were also negative for immunofluorescent staining. Thus, immunocytochemically detectable IL-1 is not present constitutively within monocytes, but rather must be induced by appropriate stimuli.

Effects of Preincubation of Antiserum with Excess Antigen. To further test the specificity of our staining reaction, each antiserum was preincubated with either the peptide used to raise that serum, or with irrelevant peptide or protein, and then used to stain cultures of stimulated mononuclear cells. An example of the results obtained is shown in Fig. 3. The ability of immune serum to stain monocytes was completely abolished by preincubation with 10 μg/ml or 50 μg/ml of relevant peptide. Preincubation of the antiserum with the same concentrations of irrelevant peptide or with 100 μg/ml of OVA had no effect on immunofluo-
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Figure 3. Effects of preincubation of antiserum with excess antigen. Antiserum against the p117-128 peptide of IL-1β was preincubated with the relevant peptide (50 μg/ml) or with p197-215 (50 μg/ml), and then used to stain LPS-/PHA-stimulated mononuclear cell cultures. (A) Indirect immunofluorescent staining with antiserum that was preincubated with irrelevant (p197-215) peptide. (B) Phase contrast micrograph of the same field shown in A. (C) Indirect immunofluorescent staining with antiserum that was preincubated with relevant (p117-128) peptide. (D) Phase contrast micrograph of the same field shown in C. Note that staining was eliminated by relevant but not irrelevant peptide. Bar, 30 μm. × 583.

These findings indicate that the observed indirect immunofluorescent staining is a result of specific antibody interactions with appropriate IL-1 determinants.

Immunoblots. To further characterize the antigenic species detected by our antisera, immunoblot experiments were carried out both on cellular lysates and on conditioned medium from mononuclear cell cultures. The results of an immunoblot analysis of cellular lysate prepared from stimulated mononuclear cells are shown in Fig. 4. After treatment with anti-p117-128 and 125I-labeled protein A, a single band with an Mr of 33,000 was observed (Fig. 4, lane 2). Control lanes treated with preimmune serum and 125I-labeled protein A (Fig. 4, lane 1) or 125I-labeled protein A alone (Fig. 4, lane 3) showed no binding. Identical results were obtained in experiments using anti-p197-215 (data not shown). No antigen was detected in unstimulated cells. Our results indicate that both antisera specifically recognize a single cell-associated species having a
molecular weight similar to that predicted by open reading frame analysis of IL-1β cDNA (6, 10).

When the same sera were used to probe immunoblots of concentrated culture supernatant from LPS- and PHA-treated mononuclear cells, an extracellular species with an apparent molecular weight of 17,500 was detected. The results of such an analysis (performed with anti-p117-128) are shown in Fig. 5. For comparison, a lane containing pure IL-1β (pI 6.8) (9) was included in the immunoblot. The major band identified in the culture supernatant has a mobility similar to that of the purified IL-1. Preimmune serum did not bind to either purified IL-1 or the concentrated culture supernatant (8).

In additional experiments not shown here, in which different amounts of monocyte lysate (18 h) were assayed in parallel with different known amounts of pure IL-1 (17.5 kD), it was estimated that ~0.3 pmol of IL-1 precursor (33 kD) was obtained from 10^5 adherent monocytes. When similar experiments were performed with culture supernatant derived from the same cells, it was estimated that, on a per cell basis, only one-half to one-fourth of the amount of IL-1 detected in the lysate was found in the culture supernatant. These findings are consistent with our observation that large amounts of immunoreactive material are found intracellularly by indirect immunofluorescence, and show that a substantial proportion of the IL-1 synthesized by stimulated monocytes remains cell-associated during the first 18 h of stimulation. Similar findings have been reported by Lepe-Zuniga and Gery (16) using bioactivity as a measure of IL-1 in cells and culture supernatants.

Immunofluorescent Staining of Intact, Unpermeabilized Cells. Our analysis of the immunofluorescent staining patterns obtained using fixed, permeabilized cells
indicated that at least most of the observed staining was intracellular. By focusing at various planes through the monocytes (which were viewed in aqueous solution and remained rounded) it was clear that fluorescence was not limited to cell surfaces, but rather extended well into the cell. Nonetheless, it was not possible in these preparations to determine whether, in addition to the internal staining, membrane fluorescence was also present. To further assess this possibility, indirect immunofluorescent staining experiments were performed on intact (living) cells so that antibody would have access only to externally exposed antigens. All antibody incubations and washes were carried out on ice and in the presence of azide to prevent antibody internalization. After completion of the immunofluorescent staining procedure, the cells were fixed in 2% paraformaldehyde and viewed as usual. Examination of cultures that had been LPS- and PHA-stimulated for 5 h, then processed as described using either antiserum against the p117-128 or the p197-215 peptide of IL-1β, revealed no immunofluorescent staining. Thus our findings with intact cells support the conclusion that the staining previously observed in fixed, permeabilized cells was indeed intracellular, but provide no evidence for the additional existence of externally exposed antigen.

Accumulation of IL-1 with Time in Stimulated Versus Unstimulated Adherent Mononuclear Cell Cultures. Because it has been reported that adhesion alone can stimulate IL-1 activity in mononuclear phagocytes (3, 4, 5, 16), we wanted to determine whether adherent but otherwise unstimulated monocytes accumu-
lated immunocytochemically detectable IL-1. In addition, it was of interest to examine the time course of IL-1 accumulation in LPS- and PHA-stimulated cultures. Accordingly, mononuclear cells in medium with or without LPS and PHA were plated onto glass coverslips and incubated at 37°C for .5, 1.5, 2.5, 3.5, 4.5, or 21 h before fixation, permeabilization, and indirect immunofluorescent staining. The results are illustrated in Fig. 6. While cells cultured in LPS and PHA showed no fluorescent staining at either .5 or 1.5 hours after plating, by 2.5 h the vast majority of monocytes were clearly IL-1+. Their fluorescent intensity, however, was relatively low. By 3.5 h the monocytes exhibited substantially more fluorescence, and the fluorescent intensity of these cells continued to increase up to 21 h. Unstimulated cells, on the other hand, behaved quite differently. As expected, at .5 and 1.5 h the cells were negative; at 2.5 h, a time when IL-1 was detectable in stimulated monocytes, unstimulated monocytes remained unstained, and by 3.5 h only an occasional cell showed weak fluorescence; at 4.5 hours, a small subpopulation of monocytes (~10%) exhibited staining. The fluorescent intensity of these cells varied from very low to fairly intense (Fig. 6 M), approaching that seen in LPS-PHA-treated cultures. Interestingly, by 21 h this staining had completely disappeared and no fluorescence was observed in the cultures. Thus, while almost all of the monocytes in LPS-PHA-treated cultures showed a striking increase in intracellular IL-1 with time, monocytes in unstimulated cultures show only a transient accumulation of IL-1 and, at the time points examined, the IL-1 was seen in only relatively few cells.

Accumulation of IL-1 within Mononuclear Cells Treated with a Single Inducing Stimulus. In all of the experiments described thus far a combination of LPS and PHA was used to stimulate cells. Our rationale for this was twofold. First, we thought it appropriate to compare activated lymphocytes with the activated monocytes in analyses of intracellular IL-1 accumulation. Second, earlier experiments had suggested that use of PHA together with LPS might enhance IL-1 production, presumably by fostering T cell-monocyte interactions (17, 18). Thus, to maximize the IL-1 produced within our cultures both PHA and LPS were routinely added to the culture medium. We have, however, carried out experiments in which each of the two stimuli was added individually. In these studies mononuclear cells were cultured in medium containing either 1 μg/ml LPS or 5 μg/ml PHA, and then fixed and permeabilized at 4.5 or 21 h postplating. Microscopic examination revealed that cultures treated with LPS alone contained very few adherent lymphocytes and that monocytes within these cultures were always almost present as single cells rather than in cell clusters. Cultures treated with PHA, on the other hand, exhibited many aggregates containing both lymphocytes and monocytes. These cultures in fact appeared virtually identical to cultures stimulated with LPS and PHA together. Immunofluorescent staining of the cultures showed that following treatment with either of the two stimuli, intense monocyte-specific fluorescence was observed at 4.5 h, and staining of even greater intensity was observed at 21 h. Antisera to the amino terminal peptide and to the internal IL-1 peptide gave identical results, and in every case almost all of the monocytes were stained. Thus, in mononuclear cell preparations, either stimulus by itself is capable of inducing the accumulation of IL-1 within monocytes.
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Discussion

The present work shows that highly specific antisera raised against synthetic IL-1 peptides can be used to localize IL-1 within stimulated human monocytes. That the observed immunofluorescent staining is indeed specific has been shown by several different lines of evidence. First, while no staining of mononuclear cell cultures was obtained using either preimmune or anti-KLH sera, antisera against either the amino terminus or an internal region of mature IL-1β produced identical staining patterns. Second, in each case intense cytoplasmic fluorescence was seen in monocytes, but not in lymphocytes or platelets present in the same cultures. Stimulation of lymphocytes with PHA did not change their staining intensity. Unstimulated monocytes did not contain immunocytochemically detectable levels of IL-1; however, after stimulation with LPS and/or PHA, fluorescent staining of monocytes became apparent in a time-dependent manner. Third, immunofluorescent staining was abolished by preincubation of antisera with relevant peptide, but was unaffected by preincubation with irrelevant peptide or protein. The fact that we could completely block staining with relevant peptide, as well as our finding that neither preimmune nor anti-KLH serum produced staining, indicates that the fluorescence observed in our fixed, permeabilized preparations was not due to antibody binding to Fc receptors. Finally, “western” analyses of cell lysates from stimulated monocytes revealed a single band having an apparent molecular weight similar to that predicted from the nucleotide sequence of IL-1β cDNA. The intensity of this 33-kD band paralleled staining intensity during response to LPS/PHA (unpublished observations, Limjuco et al.). Although we cannot yet rule out the possibility that the antisera also recognize the other species of IL-1 (IL-1α), lack of extensive amino acid sequence homology between the two species (6) suggests that the antisera are likely to be specific for IL-1β. When the amino acid sequences of human IL-1α and IL-1β are aligned to give the maximal number of identical residues (reference 6), only 2 of the 12 amino acids in p117-128 (IL-1β) are identical to corresponding amino acids in IL-1α. 4 of the 19 residues of p197-215 are identical.

It has previously been shown that various stimuli have different effects on the amounts of IL-1 activity found within cells and in culture supernatants (5, 16). Even the process of cellular adhesion to a culture substratum may influence IL-1 production. While studies have indicated that lysates of freshly isolated mouse macrophages or human monocytes do not contain IL-1 activity, a transient increase in intracellular IL-1 activity was observed after the cells were plated into culture dishes (5, 4, 5, 16). Our results are in general agreement with these
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findings. However, by using immunocytochemical techniques we have further shown that in response to plating all monocytes do not respond in a uniform manner. Although almost all of the cells appeared to have attached to the substratum after 0.5 h in culture, at the subsequent time points that we examined only a minority of the monocytes showed immunofluorescent staining and this was of variable intensity. It remains to be determined whether the cells that did not stain are late responders or whether they are refractory to stimulation by cell adhesion. In contrast, monocytes in cultures treated with LPS and PHA responded in a much more uniform manner, the vast majority showing a synchronous and progressive increase in staining with time. Furthermore, it was clear from experiments using the stimuli individually that either LPS or PHA alone was sufficient to stimulate the accumulation of high levels of intracellular IL-1. The ability of T cell mitogens to stimulate IL-1 production has previously been reported (18).

Although Kurt-Jones et al. (19) have reported the presence of membrane-associated IL-1 on stimulated murine macrophages, we have not detected surface IL-1 in our immunocytochemical experiments on stimulated human monocytes. We cannot, however, exclude the possibility of membrane IL-1 in amounts below our limits of detection or the possibility of a form of membrane IL-1 not recognized by either of our antisera. Further, while our experiments indicated that the IL-1 that we could detect was internal, and while the fluorescence observed in fixed and permeabilized cells appeared to extend throughout the monocyte cytoplasm, light microscopy does not provide sufficient resolution to determine whether or not the IL-1 is packaged in discrete subcellular organelles. Since little is now known about the storage or release of intracellular IL-1 it should prove especially interesting to examine the subcellular distribution of the IL-1 at the electron microscopic level.

The observed accumulation of IL-1 within cells, together with the apparent lack of a hydrophobic signal sequence within the precursor, suggests that IL-1 may not be exported from cells in the manner typical for secretory proteins. It is interesting to note that while almost all of the IL-1 detected in conditioned medium had an apparent molecular weight of 17.5 kD, immunoblot analysis of cell lysates revealed no mature 17.5-kD IL-1 within cells and only a single higher molecular weight band (Fig. 4, lane 2). Giri et al. have obtained similar results in immunoprecipitation experiments examining IL-1 production by murine macrophages (7). These findings suggest that IL-1 precursor is cleaved either during or shortly after release and raise the possibility that cleavage of IL-1 precursor molecules is somehow linked to externalization.

IL-1 may play a role in immune responsiveness, and also appears to be an important mediator during chronic inflammation. Furthermore, it now appears that IL-1 can be produced by other cell types in addition to monocytes (1, 20). The present works suggests that highly specific anti-IL-1 antisera may allow immunocytochemical detection of IL-1 in different cell types, such as epidermal cells (21), as well as the in situ identification and localization of IL-1-producing cells within inflammed tissue.
Summary

We have used synthetic peptides coupled to KLH to raise high titer antisera to human IL-1β, and in the present report show the usefulness of these sera for immunocytochemical analyses of IL-1 production. Using indirect immunofluorescence, we have been able to specifically identify IL-1 within human monocytes and to monitor its accumulation with time. After indirect immunofluorescent staining of LPS- and PHA-stimulated mononuclear cell cultures, intense cytoplasmic fluorescence was observed in 93% of the monocytes, but not in lymphocytes or platelets present in the same preparation. Unstimulated monocytes did not contain immunocytochemically detectable IL-1. When put into culture, however, some of the otherwise unstimulated monocytes subsequently showed a transient accumulation of intracellular IL-1. Monocytes cultured in the presence of LPS and PHA exhibited detectable fluorescence after 2.5 h, and the fluorescent intensity of these cells continued to increase over the course of 21 h. Fluorescent staining was abolished by preincubation of the sera with relevant but not irrelevant peptide, and while preimmune or anti-KLH serum produced no staining, antisera against either the amino terminus or an internal region of IL-1β produced identical staining patterns. Immunoblot analyses of lysates from stimulated monocytes showed that the antisera against IL-1 recognize a single intracellular species with an apparent molecular weight (33 kD) similar to that predicted for IL-1 precursor from the nucleotide sequence of IL-1 cDNA (6, 10). The ability to specifically identify and immunocytochemically localize IL-1 within producing cells should prove extremely useful for studying the in situ production of IL-1 in immune-based and inflammatory diseases.

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Note added in proof: We have recently tested the antisera used in the present paper on pure, monocyte-derived, human IL-1α. The sera show no crossreactivity with the IL-1α in either ELISA or immunoblot analyses.

References

1. Durum, S. K., J. A. Schmidt, and J. J. Oppenheim. 1985. Interleukin 1: an immunological perspective. Annu. Rev. Immunol. 3:263.
2. Wood, D. D., E. K. Bayne, M. B. Goldring, M. Gowen, D. Hamerman, J. L. Humes, E. J. Ihrie, P. E. Lipsky, and M. J. Staruch. 1985. The four biochemically distinct species of human interleukin-1 all exhibit similar biologic activities. J. Immunol. 134:895.
3. Unanue, E. R., and J. M. Kiely. 1977. Synthesis and secretion of a mitogenic protein by macrophages: description of a superinduction phenomenon. J. Immunol. 119:925.
4. Mizel, S. B., and D. L. Rosenstreich. 1979. Regulation of lymphocyte-activating factor (LAF) production and secretion in P388D1 cells: identification of high molecular weight precursors of LAF. J. Immunol. 122:2173.
5. Gery, I., P. Davies, J. Derr, N. Krett, and J. A. Barranger. 1981. Relationship
between production and release of lymphocyte-activating factor (interleukin-1) by murine macrophages. 1. Effects of various agents. Cell. Immunol. 64:293.
6. March, C. J., B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp, and D. Cosman. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature (Lond.). 315:641.
7. Giri, J. G., P. T. Lomedico, and S. B. Mizel. 1985. Studies on the synthesis and secretion of interleukin-1. 1. A 33,000 molecular weight precursor for interleukin-1. J. Immunol. 134:343.
8. Limjuco, G., S. Galuska, J. Chin, P. Cameron, J. Boger, and J. A. Schmidt. 1986. Antibodies of predetermined specificity to the major charged species of human IL-1. Proc. Natl. Acad. Sci. USA. In press.
9. Cameron, P., G. Limjuco, J. Rodkey, C. Bennett, and J. A. Schmidt. 1985. Amino acid sequence analysis of human interleukin 1(IL-1): evidence for biochemically distinct forms of IL-1. J. Exp. Med. 169:790.
10. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin-1 precursor cDNA. Proc. Natl. Acad. Sci. USA. 81:7907.
11. Merrifield, R. B. 1963. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85:2149.
12. Liu, F.-T., M. Zinnecker, T. Hamaoka, and D. H. Katz. 1979. New procedures for preparation and isolation of conjugates of proteins and a synthetic copolymer of D-amino acids and immunochemical characterization of such conjugates. Biochemistry. 18:690.
13. McLean, I. W., and P. K. Nakane. 1974. Periodate-lysine-paraformaldehyde fixative a new fixative for immunoelectron microscopy. J. Histochem. Cytochem. 22:1077.
14. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:680.
15. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4350.
16. Lepe-Zuniga, J. L., and I. Gery. 1984. Production of intra- and extracellular interleukin-1 (IL-1) by human monocytes. Clin. Immunol. Immunopathol. 31:222.
17. Farr, A. G., M. E. Dorf, and E. R. Unanue. 1977. Secretion of mediators following T lymphocyte-macrophage interaction is regulated by the major histocompatibility complex. Proc. Natl. Acad. Sci. USA. 74:3542.
18. Mizel, S. B., J. J. Oppenheim, and D. L. Rosenstreich. 1978. Characterization of lymphocyte activating factor (LAF) produced by the macrophage cell line P88D1. I. Enhancement of LAF production by activated T lymphocytes. J. Immunol. 120:1497.
19. Kurt-Jones, E. A., D. I. Beller, S. B. Mizel, and E. R. Unanue. 1985. Identification of membrane-associated interleukin 1 in macrophages. Proc. Natl. Acad. Sci. USA. 82:1204.
20. Oppenheim, J. J., and I. Gery. 1982. Interleukin-1 is more than an interleukin. Immunol. Today. 3:113.
21. Lugur, T. A., B. M. Stadler, B. M. Lugur, B. J. Mathieson, M. Mage, J. A. Schmidt, and J. J. Oppenheim. 1982. Murine epidermal cell-derived thymocyte activating factor resembles murine interleukin-1. J. Immunol. 128:2147.