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# INDUCTION OF INTERLEUKIN 1 $\alpha$ mRNA DURING THE ANTIGEN-DEPENDENT INTERACTION OF SENSITIZED T LYMPHOBLASTS WITH MACROPHAGES

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Among mononuclear leukocytes, macrophages are the principal source of the cytokine IL-1. Little or no production is detected in stimulated B and T lymphocytes, or in dendritic cells (1, 2). It is thought that T cells induce macrophage IL-1 production early in an immune response, and that this IL-1 is a prerequisite for the onset of T cell proliferation (3, 4). However, the analysis of IL-1 production during the APC-T cell interaction is hampered by a lack of specific neutralizing antibodies and by the fact that bioassays for IL-1 are sensitive to other polypeptides like IL-2 and IL-4 that are produced by T lymphocytes.

We have used a murine IL-1 $\alpha$  cDNA probe to look more specifically for the expression of the IL-1 gene during the macrophage-T cell interaction. A number of systems have been studied in which T cells bind to peritoneal macrophages and then begin to proliferate. In only one case, however, is IL-1 mRNA readily detected: the interaction of sensitized T lymphoblasts with antigen-bearing macrophages. These findings indicate that quantitatively, the strongest T cell-mediated signal for IL-1 production occurs in the efferent limb of a class II-restricted, immune response.

## Materials and Methods

**Mice.** 6-10-wk-old mice of both sexes were used. C57Bl/6, A, C3H/He, (BALB/c  $\times$  DBA/2)F<sub>1</sub> [CxD2 F1] were from the Trudeau Institute, Saranac Lake, NY, and CBA/J from The Jackson Laboratories, Bar Harbor, ME.

**Culture Medium.** We used RPMI 1640 supplemented with 5% FCS, 2-ME, and antibiotics.

**Cells.** Thioglycollate-elicited peritoneal macrophages were obtained 4 d after the injection of 1 ml of Brewer's thioglycollate broth intraperitoneally. About 10<sup>7</sup> macrophages were plated on each 100-mm Petri dish (No. 3001, Falcon Labware, Oxnard, CA), and the few nonadherent cells were removed 2 h later. The macrophages were cultured overnight (16 h) before use as accessory cells. In one set of the experiments (Results, Fig. 2 A), the macrophages were cultured for 3 d with 10U/ml murine rIFN- $\gamma$  to increase the expression of surface MHC products. The macrophages were cultured with different populations of T lymphocytes, mitogens (Con A, 1  $\mu$ g/ml, Sigma Chemical Co., St. Louis, MO; 2C11 anti-CD3 mAb [5], a kind gift of Dr. J. Bluestone), and/or 1  $\mu$ g/ml LPS (*Salmonella typhimurium*, Difco Laboratories Inc., Detroit, MI). Unprimed T cells were nylon wool nonadherent, Ia<sup>-</sup> spleen and lymph node. Sensitized T lymphoblasts were generated from dendritic-T cell clusters as described (6), either in the primary mixed leukocyte (MLR) or in the polyclonal response to Con A. Single large petri dishes were used for the RNA analyses, while companion microcul-

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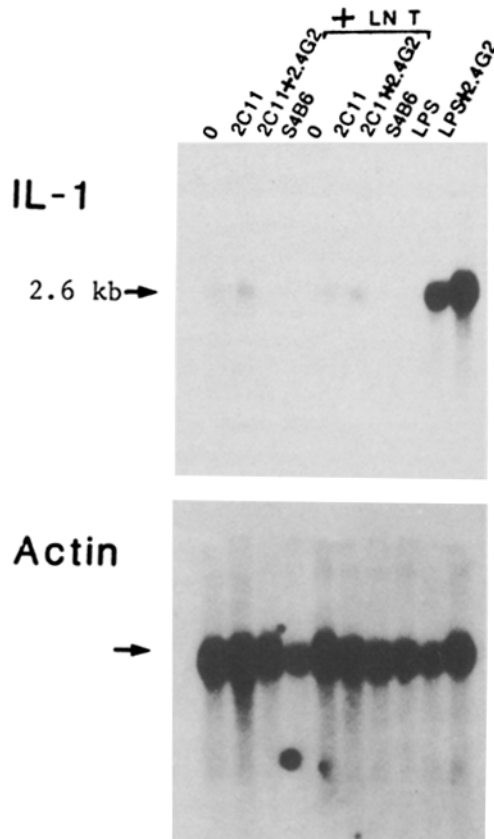


FIGURE 1. Lack of IL-1 $\alpha$  mRNA during anti-CD3 mitogenesis.  $10^7$  macrophages were cultured with the indicated mAb (2C11 hamster anti-mouse CD3; S4B6 hamster anti-mouse IL-2; 2.4G2 rat anti-mouse Fc receptor) without (four left lanes) or with (four middle lanes) an equal number of lymph node T cells. As a control, the macrophages were stimulated with 1  $\mu$ g/ml LPS  $\pm$  2.4G2 mAb (right two lanes). RNA was extracted at 4 h and hybridized with IL-1 and actin probes. In companion microcultures, the proliferation by  $3 \times 10^5$  starting T cells and  $3 \times 10^3$  macrophages at 42–48 h was 190,000 cpm in the absence of 2.4G2 and 1,500 cpm in its presence.

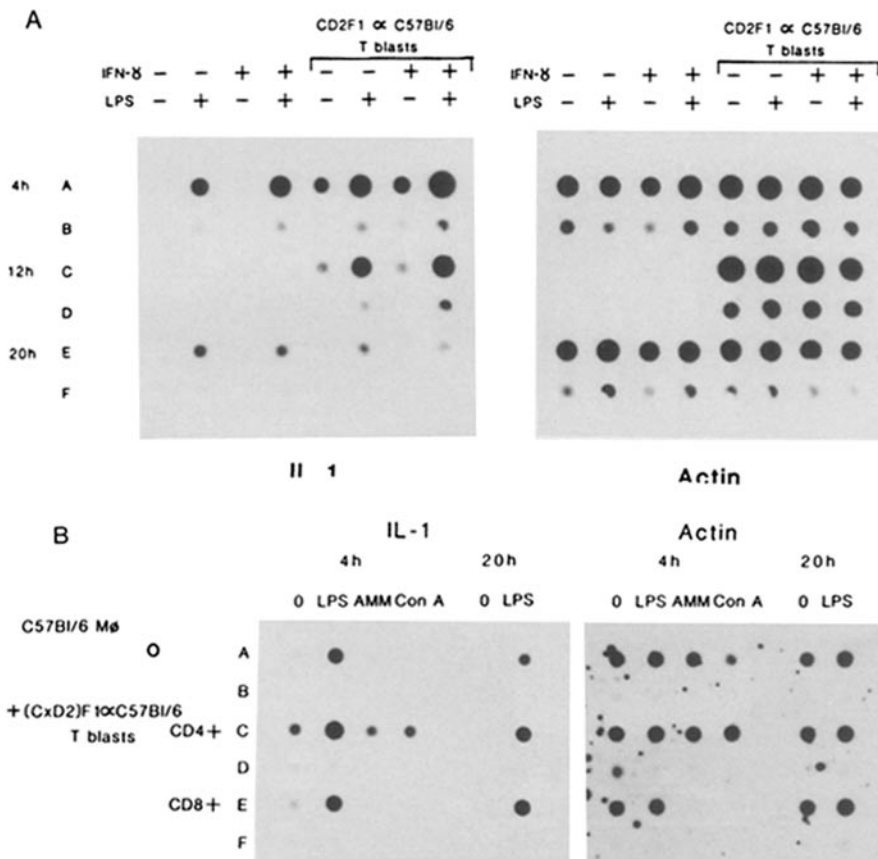
tures of  $3 \times 10^4$  T blasts or  $3 \times 10^5$  small T cells were assessed for proliferative activity. The latter were pulsed with 1  $\mu$ Ci [ $^3$ H]thymidine at times indicated in the Results.

**DNA-RNA Hybridization.** Cytoplasmic RNA, extracted with a modified phenol chloroform method, was probed with the murine IL-1 $\alpha$  cDNA probe (7), and chicken  $\beta$ -actin probe (8), as detailed (1). We dislodged most of the T cells and then analyzed the adherent macrophages and T cells separately. Typically, a plate of  $10^7$  macrophages yielded 20  $\mu$ g of RNA. We probed this RNA after separation on 1.5% agarose gels (1–2  $\mu$ g; Northern blot) or application to Gene Screen (New England Nuclear, Boston, MA; 5 and 0.5  $\mu$ g, dot blotting).

## Results

**Lack of IL-1 mRNA During Primary, Polyclonal T Cell Responses.** We had previously studied lectin-induced T cell proliferation with peritoneal macrophages as accessory cells. No IL-1 $\alpha$  mRNA was detected (1). We extended the analysis by studying several time points (4, 12, 20 h; not shown) and by studying another polyclonal stimulus, the 2C11 anti-CD3 mAb isolated by Leo et al. (5). Again, no IL-1 message was detected when RNA was extracted at 4 h of this vigorous polyclonal response (Fig. 1). As a control, LPS was added and a strong signal for IL-1 mRNA was evident (Fig. 1, right lanes). These results show that IL-1 gene expression is not a prominent feature of primary, macrophage-mediated polyclonal T cell responses.

**Induction of IL-1 mRNA by Alloreactive T Blasts.** Different results were obtained



**FIGURE 2.** IL-1 $\alpha$  mRNA induction by alloreactive T blasts. (A) Time course of the response.  $10^7$  adherent macrophages were cultured for 3 d with or without 10 U/ml rIFN- $\gamma$ . The macrophages were washed and mixed with alloreactive T blasts at a ratio of 3:1 with or without 1  $\mu$ g/ml LPS as indicated for 4, 12, or 20 h before extraction of RNA. The top row of each pair had 10 times the dose of RNA. For the 12-h time point, we did not analyze macrophages in the absence of T blasts. In companion microcultures, the proliferation by  $3 \times 10^4$  starting T blasts at 20–24 h was 60,000 and 72,000 cpm with  $10^4$  of the IFN- $\gamma$  untreated or treated macrophages.

(B) Comparison of CD4 and CD8 blasts. Cx2D2 anti-C57Bl/6 T blasts were obtained from the primary MLR (6), and the CD4 and CD8 subsets were enriched by depletion with mAb to Lyt-2 (HO 2.2) and L3T4 (GK 1.5) and rabbit serum, respectively. T blasts were added to  $10^7$  macrophages at a ratio of 1:14 and cultured 4 or 20 h with no further stimuli, or with  $\alpha$ -methyl mannoside ( $\alpha$ MM), LPS, or Con A as shown. The top dose of each pair had 10 times the dose of RNA. For the 4-h time point we did not analyze the CD8 $^+$  cells in the presence of Con A or  $\alpha$ MM. In companion microcultures, the proliferation of  $1.5 \times 10^4$  blasts to  $10^4$  macrophages was 29,000 cpm for CD4 $^+$  blasts and 31,000 for CD8 $^+$ ; backgrounds were <2,000 cpm.

in an antigen-specific response, the MLR. Because peritoneal macrophages do not induce a primary MLR, we studied their interaction with freshly sensitized T blasts for which macrophages are potent APC (6, 9). Alloreactive CD4 $^+$  T blasts were generated in a primary MLR using C57Bl/6 (H-2 $^b$ ) dendritic cells and Cx2D2 (H-2 $^d$ ) T cells. The T blasts were isolated at day 4 and added to C57Bl/6 macrophages. At 4, 12, and 20 h, the cultures were separated into adherent and nonad-

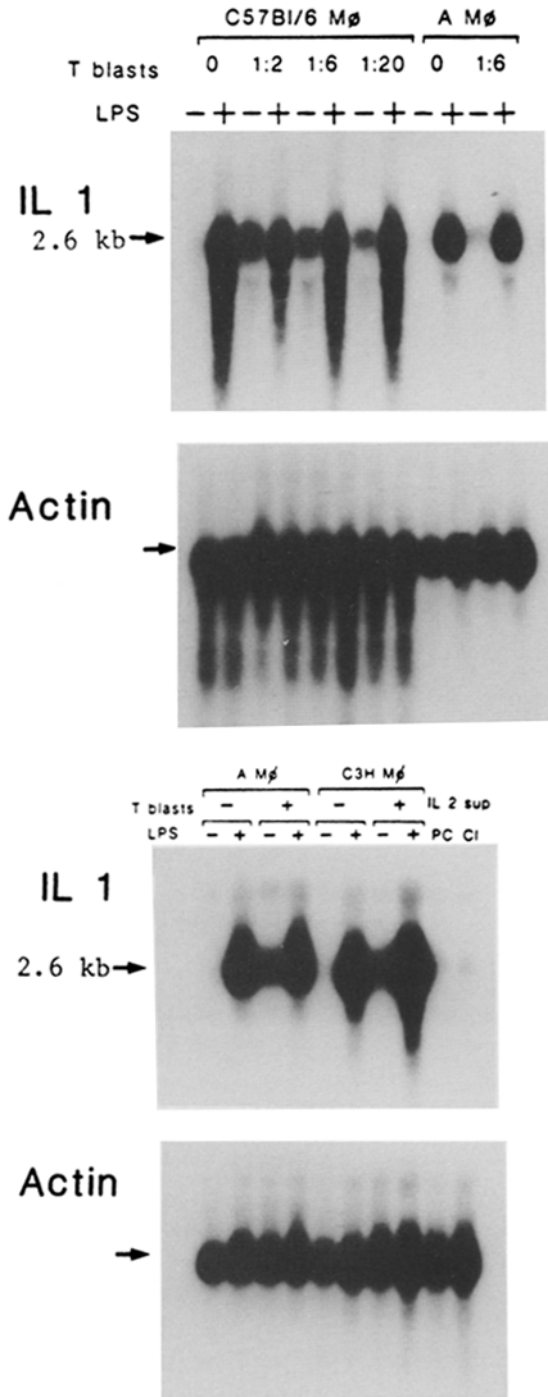


FIGURE 3. Specificity of the T blast-macrophage interaction. (A) Comparison of first-party and third-party macrophages. H-2<sup>d</sup> T blasts were induced by C57BL/6 dendritic cells and added to  $10^7$  macrophages in graded doses, as indicated above the lanes. The macrophages were from C57BL/6 [first-party] or A [Ia<sup>k</sup>, third-party] mice. RNA was isolated 4 h later. In companion microcultures, the proliferative responses of  $3 \times 10^4$  T blasts and  $10^4$  macrophages was 68,000 cpm for C57BL/6 and 4,200 cpm for strain A macrophages at 18-24 h.

(B) Comparison of two strains of MHC-matched macrophages with T cell-conditioned medium. H-2<sup>d</sup> T blasts were sensitized to A strain dendritic cells and cultured with A or C3H/He macrophages (both Ia<sup>k</sup>). In companion microcultures, the proliferation of  $3 \times 10^4$  blasts to  $10^4$  macrophages was 101,000 and 61,000 cpm, respectively, at 18-24 h. Two sources of IL-2-rich, conditioned medium were compared with the T blasts in the right two lanes: PC or polyclonal rat-conditioned medium (Collaborative Research) or 2-d medium from a dendritic-CD4<sup>+</sup> T cell MLR.

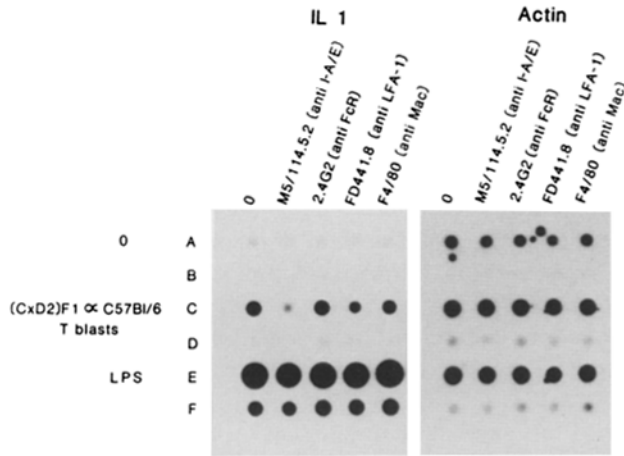


FIGURE 4. Anti-Ia mAbs inhibit the induction of IL-1 mRNA by T blasts.  $10^7$  macrophages were cultured with the indicated mAbs added as hybridoma culture supernatants at 10% vol/vol. The macrophages were stimulated with nothing (A, B),  $10^6$  alloreactive T blasts (C, D), or 1  $\mu$ g/ml LPS (E, F), with the top row in each pair representing a 10-fold greater dose of RNA. The proliferation at 18–24 h of companion cultures of  $3 \times 10^4$  blasts and  $10^4$  macrophages was 61,000 (control); 4,800 (anti-Ia); 84,000 (F4/80); 78,000 (2.4G2); and 33,000 (LFA-1) cpm.

herent fractions containing the macrophages and T blasts respectively, and the RNA was extracted. IL-1 mRNA was induced, but only in the adherent macrophages (not shown). In all four experiments, the peak levels were early at 4 h, and IL-1 mRNA was no longer detectable at 20 h (Fig. 2 A). Since class II MHC antigens seem necessary to mediate the macrophage–T cell interaction in these cultures (see below), we tried to enhance the response by upregulating the levels of macrophage Ia with IFN- $\gamma$ . However, IFN- $\gamma$  pretreatment had little effect (Fig. 2 A). By FACS analysis, addition of IFN- $\gamma$  increased macrophage staining with FITC–anti-Ia mAb from twice background to four times background (data not shown). As a positive control, replicates of all cultures were challenged with LPS. In each case, a strong IL-1 mRNA signal was noted that was two to three times more intense than the signal induced by T blasts (Fig. 2 A).

Alloreactive CD4<sup>+</sup> and CD8<sup>+</sup> blasts were then compared. Only the CD4<sup>+</sup> blasts were active, and again the response was clear at 4 h but not detectable at 20 h [Fig.

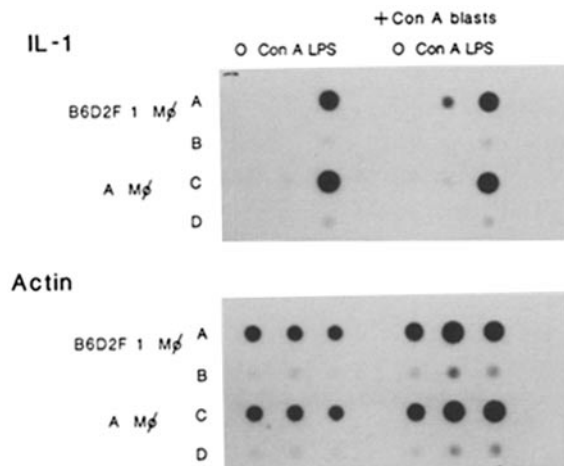


FIGURE 5. Weak IL-1-inducing activity of polyclonal T blasts. T blasts were induced with Con A in B6xD2 F1 CD4<sup>+</sup> T cells. The blasts were washed with  $\alpha$ MM and added to syngeneic or allogeneic (A strain) macrophages with no additional stimulus (0), Con A, or LPS. The T blasts proliferated actively in each case, i.e.,  $3 \times 10^4$  blasts and  $10^4$  macrophages gave responses in the presence of Con A of 100,000 (B6D2) and 111,000 (A) cpm at 18–24 h. The top of each pair has a 10-fold greater dose of RNA.

2 B]. The addition of Con A did not increase the levels of IL-1 mRNA for either subset of T blasts. A strong response to LPS was observed in all the macrophages,  $\pm$  CD4<sup>+</sup> or CD8<sup>+</sup> blasts (Fig. 2 B).

*Immunologic Specificity of the Macrophage-T Blast Interaction.* If (BALB/c  $\times$  DBA/2)F<sub>1</sub> CD4<sup>+</sup> T cells [Ia<sup>d</sup>] were sensitized to C57Bl/6 dendritic cells [Ia<sup>b</sup>], the lymphoblasts induced IL-1 mRNA in C57Bl/6 but not in third-party, A strain [Ia<sup>k</sup>] macrophages [Fig. 3 A]. The third-party cells did respond to LPS, however. The response of the Ia<sup>b</sup> macrophages was proportional to the dose of T blasts (Fig. 3 A).

When the H-2<sup>d</sup> T cells were sensitized to strain A instead of C57Bl/6, then IL-1 was induced in macrophages from both Ia<sup>k</sup> strains that we tested, A and CBA/J (Fig. 3 B). Induction of IL-1 seemed to require a direct T blast-macrophage interaction, since a variety of T cell-conditioned media did not induce IL-1 directly (Fig. 3 B, right lanes).

To verify that the T blasts had to recognize macrophage Ia antigens to induce IL-1 mRNA, a panel of mAb was tested for blocking activity. An mAb, M5/114, that recognizes I-A and I-E products in H-2<sup>d</sup> mice (10), markedly reduced cell proliferation and the induction of IL-1 mRNA (Fig. 4). The FD441.8 mAb to the T cell adhesion molecule LFA-1 $\alpha$  (11) also reduced the IL-1 $\alpha$  mRNA signal, but two other mAbs, F4/80 (12) and 2.4G2 (13), to the macrophage did not [Fig. 4]. None of the mAb reduced the IL-1 response to LPS [Fig. 4].

Polyclonal T blasts, induced with the lectin Con A, were then compared with allospecific cells. Although the two types of blasts were prepared in the same way from dendritic-T cell clusters, and proliferated actively upon rechallenge with macrophages, very little induction of IL-1 mRNA was observed with the polyclonal blasts. The strongest IL-1 signal we observed in four experiments is shown in Fig. 5, and was noted with syngeneic (B6xD2F1) but not allogeneic (A strain) macrophages. Since both strains of macrophages were equally active as accessory cells for the proliferation of the T blasts (legend, Fig. 5), it is possible that induction in syngeneic macrophages might occur through self-MHC-restricted T cells that had previously been expanded in the T cell donor strain. Taken together, the data in Figs. 1, 3, 4, and 5 indicate that quantitatively the induction of IL-1 mRNA is greatest when there is a class II MHC-restricted interaction of macrophages with sensitized CD4<sup>+</sup> T blasts.

### Discussion

Prior studies of IL-1 production during the interaction of macrophages and T lymphocytes have emphasized a single theme. Resting T cells recognize antigen on the macrophage surface ("signal one"), the macrophage is induced to make IL-1, and then the T cell uses this IL-1 ("signal two") to begin to grow. It has been difficult to obtain direct evidence for this hypothesis with primary populations of T cells (14, 15). We have used an IL-1 $\alpha$  cDNA probe to specifically monitor IL-1 gene expression when macrophages are acting as accessory cells for unprimed and freshly sensitized T blasts. In each case (lectin, anti-CD3 mAb, alloreactive T blasts), the T cells bound to the macrophages and underwent a large proliferative response. Yet in only one case was a strong IL-1 mRNA signal observed, when sensitized T cells were being rechallenged with antigen-bearing macrophages. Alternative techniques, such as in situ hybridization for IL-1 mRNA, may reveal some IL-1 during the interac-

tion of macrophages with T cells in polyclonal systems. However, the antigen-specific T blast is quantitatively the strongest stimulus.

Hybridization to RNA from bulk cultures does not distinguish between IL-1 production by macrophages or T cells, but we suspect that the macrophage is the source. In all experiments we extracted RNA both from macrophages that remained adherent and the nonadherent T cells. Only the former contained IL-1 mRNA. Bhardwaj, N., et al. (manuscript in preparation) used an immunofluorescence method to localize IL-1 $\beta$  during the macrophage-T cell interaction. Again, macrophages and not T cells, contained IL-1.

Our findings suggest that macrophage IL-1 production during cell-mediated immunity is quantitatively greatest after rather than before the T cell has become sensitized to antigen on presenting cells. It is of interest that IL-1 synergizes with IL-4, but not with IL-2, in inducing the growth of Th2 lymphocyte clones (16). By inducing IL-1, blasts that secrete IL-4 would show an enhanced proliferative response. In this scenario, IL-1 would act as a "second signal" for the growth of some T lymphoblasts but would have less of a role to play as a T cell-activating factor early in a primary response, or in the sustained growth of IL-2-producing T blasts (14).

### Summary

DNA-RNA hybridization with an IL-1 $\alpha$  cDNA probe was used to monitor the induction of IL-1 in macrophages that were acting as accessory cells for the proliferation of T lymphocytes. Mouse peritoneal macrophages bound and stimulated T lymphocytes in the presence of the mitogens, Con A, or anti-CD3 mAb, but little or no IL-1 mRNA was detectable. In contrast, if the T cells were first sensitized in a mixed leukocyte reaction with dendritic cells and then added to macrophages, IL-1 mRNA was clearly induced. Induction of the IL-1 $\alpha$  gene seemed to require the recognition of class II MHC products on the macrophage because of the following observations: specific rather than third-party macrophages were responsive to the T blast but not to T cell-conditioned media; induction was blocked by an anti-Ia mAb; CD4<sup>+</sup> rather than CD8<sup>+</sup> blasts were active; and polyclonal Con A blasts were much less efficient than antigen-specific T cells. Our data indicate that the strongest signal for IL-1 production during the macrophage-T cell interaction occurs in the efferent limb of the response, after rather than before the formation of class II MHC-restricted T lymphoblasts.

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