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# Interleukin 1 can replace the requirement for I-A-positive cells in the proliferation of antigen-primed T cells

(T cell activation/adherent cells/Ia antigens)

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**ABSTRACT** **Antigen-primed T cells have been shown to require I-region-compatible adherent cells, as well as the priming antigen, to proliferate *in vitro*. We postulated that the Ia-recognition event is required for the T cell to induce secretion of the monokine interleukin 1 (IL 1) from adherent cells; the conventionally held view is that Ia is directly required for T cell activation. Our hypothesis predicts that IL 1 could replace the requirement for $Ia^+$ cells in T cell proliferation assays *in vitro*. To test this prediction, we depleted keyhole limpet hemocyanin (KLH)-primed C57BL/6 mouse lymph node cells of $I\text{-}A^+$ cells by treating with monoclonal anti-$I\text{-}A^b$ and complement. As expected, this treatment eliminated the ability of KLH to provoke a proliferative response by primed T cells. Proliferation was restored by providing exogenous IL 1, but only in conjunction with added KLH. The proliferative response of primed T cells could also be blocked by adding anti-$I\text{-}A^b$ to culture, and this inhibition could similarly be reversed by providing IL 1 in the presence of the specific antigen KLH. On the basis of these findings we propose a model of T cell activation and discuss its implications.**

The *in vitro* expression of most T lymphocyte activities requires the presence of nonlymphoid cells with the property of glass adherence (1, 2). To exert supportive effects, the adherent cell population must contain a subset of cells bearing Ia antigens of the same haplotype encountered by the T cell during ontogeny (3–6). Ia molecules on the adherent cell membrane are generally thought to satify an "antigen-presentation" requirement for T cell activation, occurring when T cells simultaneously bind Ia and "nominal" antigen on the adherent cell surface. The adherent cell population also provides a second requirement for T cell activation, the monokine interleukin 1 (IL 1) (7–9).

We propose an alternative interpretation of the Ia requirement in T cell activation: rather than being directly required (together with antigen) to trigger T cells, Ia recognition could be required for T cells to trigger adherent cells to secrete IL 1. Therefore, the minimal number of signals acting directly on the T cell would be two: antigen and IL 1. This hypothesis was founded on our observations that activated T cells elicit IL 1 secretion from adherent cells, that this cellular interaction is restricted by polymorphisms encoded by genes in the *I* region of the H-2 complex [also observed by Farr *et al.*, (10)], and that this interaction is also blocked by monoclonal antibodies to I-A (unpublished observations).

We then examined several T cell activities known to require the presence of $Ia^+$ adherent cells *in vitro*, including help in the primary humoral response, priming of helper cells, and concanavalin A (Con A)-induced proliferation. We depleted cultures of adherent cells by passage through Sephadex G-10, and we depleted cultures of I-A-positive cells by anti-I-A and complement (C), anti-I-A blocking, or genetic strategies using radiation chimeras. In every case, cultures depleted of these cell populations were restored by IL 1 (unpublished observations).

In the present study, we examine the requirement for $I\text{-}A^+$ cells, IL 1, and antigen to induce proliferation of primed T cells *in vitro*. We demonstrate that IL 1 can replace $I\text{-}A^+$ cells and restore the proliferative response in cultures blocked by anti-I-A, but it must act in concert with the specific priming antigen.

## MATERIALS AND METHODS

**Mice.** C57BL/6J males, 7–10 weeks of age, were purchased from The Jackson Laboratory.

**Immunization and Proliferation Assay.** The method of Corradin *et al.* (11) was followed. Mice were immunized at the base of the tail with 100 μg of keyhole limpet hemocyanin (KLH) (Calbiochem) in complete Freund's adjuvant (CFA). Ten to 15 days later, draining lymph nodes from 5–10 mice were removed and cells were centrifuged on discontinuous Percoll (Pharmacia) gradients to remove blast cells. Small lymphocytes were treated with monoclonal anti-$I\text{-}A^b$ (1:500 dilution) and rabbit C. Cells ($5 \times 10^5$) were cultured in amino acid-rich medium (11) supplemented with 0.5% fresh syngeneic serum and 50 μM 2-mercaptoethanol in microtiter wells (Costar, Cambridge, MA). KLH (100 μg/ml), IL 1 (0.5%), and anti-$I\text{-}A^b$ (1:500 final dilution) were added to cultures. Four days later proliferation was assayed by pulsing with 1 μCi ($3.7 \times 10^4$ becquerels) of [$^3$H]thymidine (New England Nuclear) for 4 hr and harvesting with a MASH II apparatus (Microbiological Associates, Bethesda, MD).

**Monoclonal Antibodies.** Hybridoma clone Y8P was a generous gift of C. A. Janeway and P. Conrad. The monoclonal product (purified from ascitic fluid by precipitation with ammonium sulfate) is specific for $I\text{-}A^b$ antigen as demonstrated by strain distribution and by precipitation of the $A^b_\alpha$ $A^b_\beta$ complex (C. A. Janeway, personal communication). Monoclonal antibodies specific for Lyt-1.2 and Thy-1.2 antigens were generously provided by F. W. Shen.

**IL 1.** Cultures of $P388D_1$ cells (12, 13) were stimulated 4 days with *Escherichia coli* lipopolysaccharide (Difco) at 20 μg/ml. Supernates were precipitated with 65% saturated ammonium sulfate, dialyzed, then fractionated on a column of Sephadex G-75 superfine (Pharmacia). Biologic activity (assayed by thymocyte proliferation) was contained in the 15,000- to 20,000-dalton fractions. Active fractions were pooled and concentrated on Amicon UM10 filters. The final concentration of IL 1 used in

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Abbreviations: C, complement; CFA, complete Freund's adjuvant; Con A, concanavalin A; IL 1, interleukin 1; KLH, keyhole limpet hemocyanin.

these experiments corresponds to approximately 50 units/ml (14).

## RESULTS

Mice were primed to KLH and cells from the draining lymph nodes were fractionated on Percoll gradients; the small lymphocyte fractions were then rechallenged with KLH *in vitro*. Blast cell fractions were not used because preliminary studies (data not shown) indicated that IL 1, independent of added KLH, would drive them to proliferate. However, DNA synthesis by primed small lymphocytes, as shown in Fig. 1, depends on added KLH; IL 1 alone has no effect (in the two experiments shown, proliferation in C-treated control cultures given KLH was not augmented by adding IL 1, but such a facilitation is occasionally seen). When primed cells are treated with monoclonal anti-I-A and C prior to culture, their ability to proliferate in response to KLH is lost, but it is restored by the addition of IL 1. In the seven experiments that have been performed to date, responses inhibited by anti-I-A and C have increased an average of 29-fold in the presence of IL 1 and have achieved an average level of 70% of the magnitude of control responses.

The effect of adding anti-I-A to cultures demonstrates that the I-A structure is more than just a marker on a required cell; it actually participates in inducing proliferation. This can be inferred from the data in Fig. 2, which shows two typical experiments demonstrating that "blocking" with anti-I-A inhibits the proliferative response to KLH. This inhibition, while never as complete as that brought about by pretreating cells with anti-I-A and C, is potent nonetheless; inhibition is reversed by addition of IL 1, but again only in the presence of KLH.

Several features of the cells that respond to IL 1 replacement of I-A$^+$ cells are shown in Table 1. IL 1 does not reconstitute anti-I-A-depleted populations that are further treated with monoclonal antibodies directed against either Lyt-1.2 or Thy-1.2 determinants; therefore, T cells are required. The data in Table 1 also show that lymph node cells must be previously primed to KLH to be driven into division by IL 1 and KLH, because CFA-primed cells are not significantly stimulated by IL 1 plus KLH.

Table 1. Phenotype and priming requirement of I-A$^-$ cells proliferating in response to IL 1 plus KLH

| Priming antigen | Cell treatments before culture | [$^3$H]Thymidine incorporation with additions to culture, cpm: No additions | KLH | IL 1 | KLH + IL 1 |
|---|---|---|---|---|---|
| KLH | — — + C | 649 | 40,160 | 2,288 | 58,122 |
| | αI-A — + C | 223 | 366 | 667 | 10,436 |
| | αI-A + αLyt-1.2 + C | — | — | — | 250 |
| | αI-A + αThy-1.2 + C | — | — | — | 599 |
| CFA | | 169 | 469 | 528 | 1,102 |

## DISCUSSION

This study clearly shows that IL 1 will replace I-A$^+$ cells in antigen-driven T cell proliferation. Our results appear to contradict those of Mizel and Ben-Zvi (15), who used a similar strategy and failed to restore proliferation with IL 1 after treating the immune cells with anti-macrophage serum and C. Many technical differences could account for this disparity, but one likely candidate is our removal of low-density cells, presumably blasts, prior to culture. The IL 1 concentrations that are required to replace I-A$^+$ cells tend to induce proliferation in blast cell fractions independent of added KLH; removal of blasts therefore enabled us to use higher concentrations of IL 1 to replace I-A$^+$ cells while maintaining the KLH dependency so evident in our results. A second fundamental difference between our study and theirs lies in our methods of depleting cultures of "accessory" cells. Whereas they chose nylon wool and anti-macrophage sera, we used only monoclonal anti-I-A. Thus

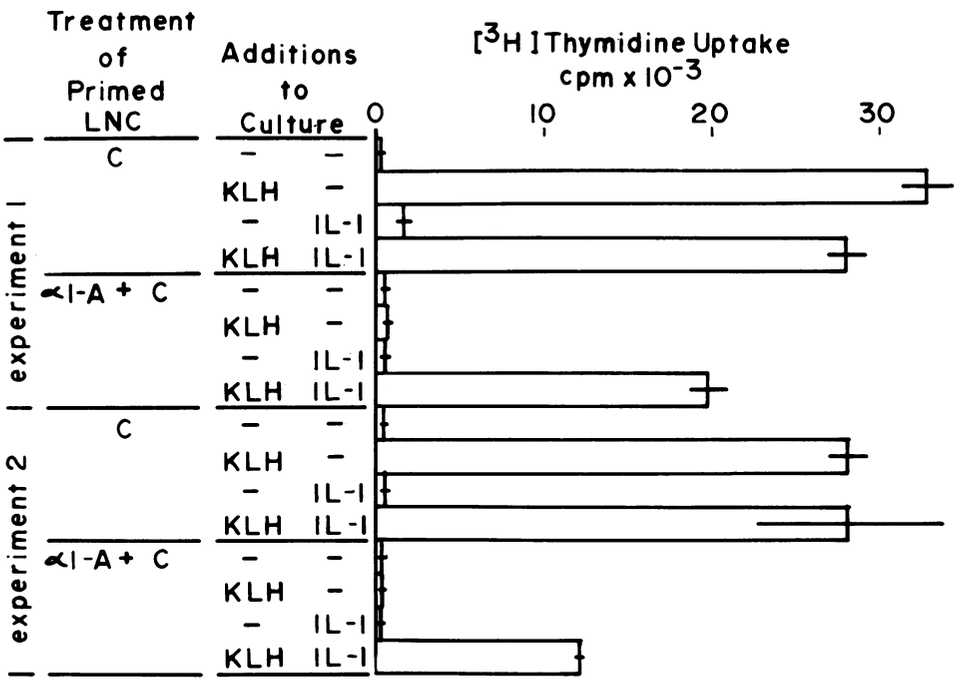


FIG. 1. IL 1 restoration of proliferation in primed lymph node cells treated with anti-I-A plus C (α = anti-). Mice were primed with KLH. Thirteen days later draining lymph node cells (LNC) were prepared, depleted of blasts, then treated with anti-I-A$^b$ and C or C alone. Cells were then cultured for 4 days in the presence of KLH and IL 1 and assayed for thymidine uptake. Two experiments are shown. Data are expressed as mean and SEM of triplicate cultures.

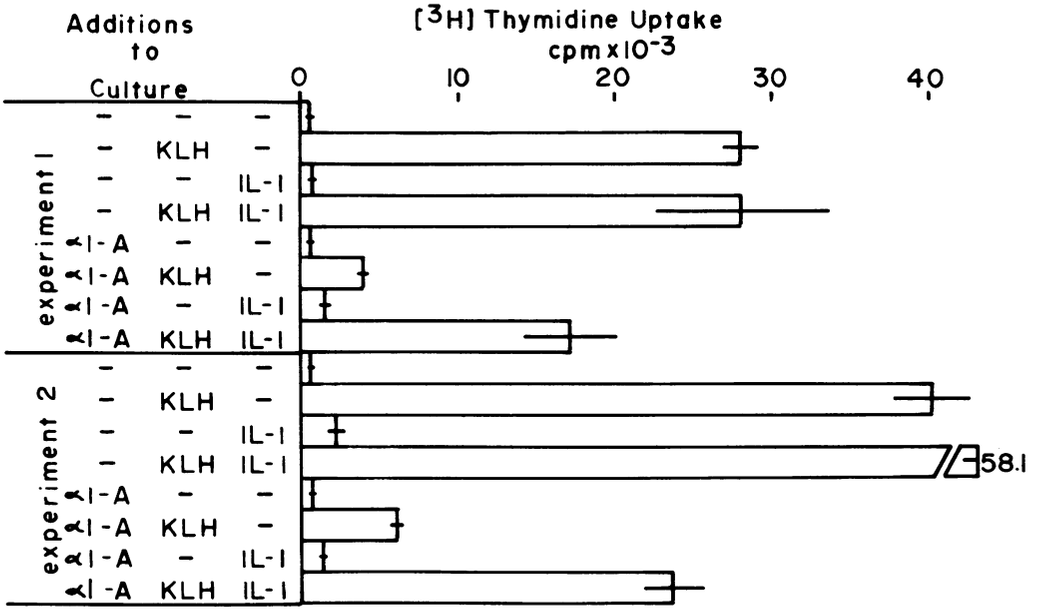


FIG. 2. IL 1 restoration of proliferation in cultures blocked with anti-I-A. Mice were primed with KLH. Thirteen days later draining lymph node cells were prepared and depleted of blasts. Cells were then cultured for 4 days in the presence of anti-I-$A^b$, KLH, and IL 1 and assayed for thymidine uptake. Two experiments are shown. Data are expressed as mean and SEM of triplicate cultures.

our study is addressed to the requirement for I-A-bearing non-T cells (which we show can be replaced by IL 1); our experiments do not examine potential requirements for $I\text{-}A^-$ adherent cells.

It could be argued that we have failed to remove residual $I\text{-}A^+$ antigen-presenting cells. However, because the proliferative response is completely abrogated by anti-I-A plus C, this argument must take the form that the two conventionally viewed roles of adherent cells, antigen presentation and IL 1 secretion, are satisfied by quite different densities of $I\text{-}A^+$ cells, antigen presentation being accomplished by remarkably few cells, IL 1 secretion requiring many. Mindful that such alternative interpretations exist, we shall discuss some implications of the theory that the fundamental requirement for I-A recognition in T cell activation is the stimulation of IL 1 secretion.

Fig. 3 illustrates our conception of events leading to the proliferation observed in primed lymph node cells. In step 1, an antigen-specific T cell encounters an adherent cell that bears antigen and I-A. We propose that this T cell becomes activated by antigen independent of I-A recognition, but whether an adherent cell is actually required to display or process antigen is unresolved. Once activated by antigen (or perhaps the continuous presence of antigen is required) the T cell uses its receptors for self-I-A to stimulate the adherent cell, which may respond in many ways, one being secretion of IL 1. Then, in step 2, IL 1 and antigen trigger a T cell (which may or may not be the same T cell shown in step 1) to "*trans*-stimulate" (16, 17) bystander T cells.

The T to adherent cell signal shown in step 1 might occur (*i*) directly through the I-A molecule (with or without a receptor complexed to the nominal antigen), (*ii*) indirectly, for example, via lymphokines as has been described (18, 19), or (*iii*) both.

The possibility of I-A molecules serving a role as signal transducers is an attractive one, based on a broad range of indirect evidence gathered by ourselves and others. We have found (unpublished observations) that monoclonal anti-I-As not only can block the antigen "specific" T cell-dependent activation of adherent cells to secrete IL 1 but also can block "nonspecific" activation such as by T cells plus Con A, or even by lipopolysaccharide. Indeed, others have observed blocking by anti-Ia sera of Con A-induced proliferation (20, 21), of Con A-induced production of T cell replacing factor (22), and of B cell mitogenesis induced by lipopolysaccharide (23), all of which support the concept of signal transduction by Ia molecules. Perhaps Ia-like molecules originally served as recognition structures for

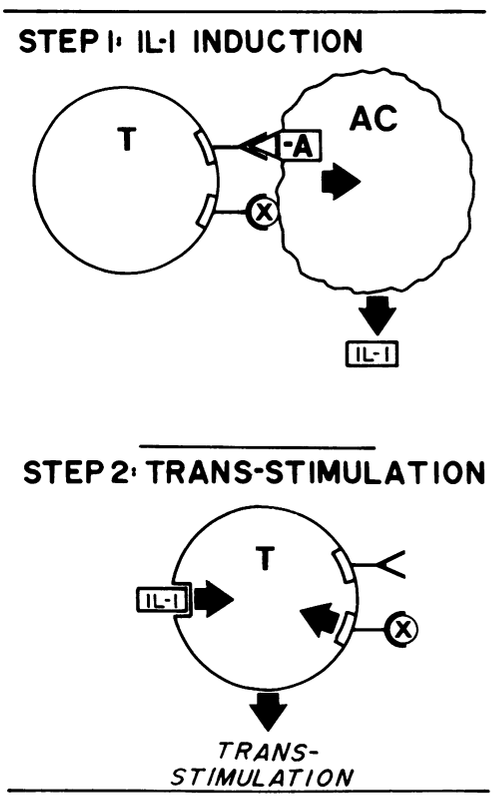


FIG. 3. Two-step model of T cell activation. In step 1, a T cell uses its receptor for I-A (and possibly for the antigen X) to activate an adherent cell (AC), which then secretes IL 1. In step 2, two signals, one from IL 1 and one from the antigen receptor, activate a specific T cell to *trans*-stimulate bystander lymphocytes.

microbial products and later in phylogeny T cells may have exploited their transducer potential.

The transducer hypothesis of Ia function can be extended to describe the thymic selection mechanism for self-recognizing T cells (24). The thymic environment may cause thymocytes to become "activated"—perhaps by a process similar to that by which antigen activates peripheral T cells to become IL 1 inducers. Only those activated thymocytes with the appropriate self-recognition capacity could induce thymic epithelial cells (transduced by their H-2 glycoproteins) to secrete growth factors into their immediate environment, thus expanding the appropriate clones of T cells [a similar selection model has been proposed by Janeway and Jason (25)].

A further extension of the transducer principle can be applied to the mechanism of immune response gene defects; thus certain antigenic determinants may be unable to participate in, or may block, the transduction process.

The present study also provides clear evidence for "dual receptor" (as opposed to "altered self") models of T cell receptors (25, 26) because KLH is recognized in the apparent absence of I-A molecules. We show that KLH alone will not induce proliferation, but it may nonetheless produce major changes in the T cell, including induction of IL 1 receptors, perhaps by driving cells from $G_0$ into $G_1$ phase—i.e., blastogenesis. Support for this hypothesis is provided by studies of the cell cycle requirements for IL 1 in lectin-driven proliferation (27, 28). Thus, blastogenesis is induced by lectins in the absence of adherent cells, and IL 1 is required later, for entry into S phase. We do not mean to imply, however, that it is IL 1 itself that drives a given blast into S phase, because one major consequence of IL 1 action on activated T cells is interleukin 2 secretion (8, 29); it is this secondary lymphokine which, for most blasts, may be the trigger for DNA synthesis.

Whatever the pathway may be that leads from IL 1 to T cell proliferation, our observation that $I\text{-}A^+$ cells can be replaced by the monokine IL 1 requires that a novel interpretation (not necessarily ours) of the role of I-A molecules in immune activation be considered.

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