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Involvement of Gamma Interferon in Antibody Enhancement by Adjuvants

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In a previous study the adjuvant action of a monophosphoryl lipid A, a nontoxic derivative of endotoxic lipopolysaccharide (LPS), was found to be negated by a monoclonal anti-gamma interferon (anti-IFN-γ) antibody. The present investigation centered on three other adjuvants of diverse microbial origins, testing for their capacity to affect the release of IFN-γ as an explanation for their antibody-enhancing action. The adjuvant action of each of the three, a wild-type LPS, synthetic poly(A)-poly(U) complexes, and a synthetic muramyl dipeptide, *n*-acetylmuramyl-L-alanyl-D-glutaminyl-*n*-butyl ester (murabutide), was transferable by adjuvant-stimulated T cells to normal spleen cells on coculture. Supernatant fluids from these T cells contained increased levels of IFN-γ. Addition of a monoclonal anti-IFN-γ antibody to adjuvant-stimulated spleen cell cultures reduced the adjuvant action by approximately one-half. Removal of natural killer cells from spleen cell populations prior to culture with antigen had no effect on the enhancement induced by LPS and monophosphoryl lipid A. It was concluded that the enhancement induced by the adjuvants LPS, poly(A)-poly(U), and murabutide is mediated in part by their action on T cells resulting in release of IFN-γ suggesting activation of a common transmembrane signal.

The cascade of mediators released following adjuvant stimulation of the immune response needs definition with respect to their sequence of appearance and spheres of influence. Recently, we demonstrated in aging mice the strong adjuvant action of monophosphoryl lipid A (MPL), a nontoxic derivative of bacterial lipopolysaccharide (LPS), and presented evidence that T cells of the helper/inducer phenotype were capable of mediating its enhancing action (40). In addition, supernatant fluids from MPL-treated T cells were effective in augmenting antibody production. An active principle in such supernatant fluids was demonstrated to be gamma interferon (IFN-γ), since the adjuvant action was ablated by a monoclonal anti-IFN-γ antibody (40). Inasmuch as this previous study implicated IFN-γ as an obligatory mediator of MPL action, we tested the role of this lymphokine in the activity of several other well-characterized adjuvants of microbial origin, i.e., a wild-type endotoxic LPS (31), the synthetic poly(A)-poly(U) complex (16), and a synthetic muramyl dipeptide, *n*-acetylmuramyl-L-alanyl-D-glutaminyl-*n*-butyl ester (murabutide) (24). Despite the well-documented adjuvant activities of these compounds, the mechanisms by which they increase antibody formation have not been delineated. All three have been shown under various conditions to affect multiple cells involved in the immune response, including T cells, B cells, natural killer (NK) cells, and macrophages, through stimulation of cellular proliferation and of secretion of various monokines and lymphokines. Identification of the initial target cell and cytokine release setting the cascade in motion, however, has been elusive. The results of the study described herein suggest that an early amplification of IFN-γ levels, resulting in an increase in the number of antibody-forming cells, is important for the action of all three of these adjuvants.

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MATERIALS AND METHODS

Mice. BALB/c mice were either purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) or bred from Charles River breeding pairs in our animal facility. Male and female mice were used at 2 to 6 months of age.

Antigen. Defibrinated sheep erythrocytes (SRBC) (Kroy Medical, Stillwater, Minn.) were used as the antigen. They were washed three times with Hanks balanced salt solution (HBSS), diluted in Click medium containing 10% fetal bovine serum (FBS) to 4×10^8 cells per ml, and added to spleen cell cultures in a 25-μl volume (10^7 cells per ml).

Adjuvants. LPS from *Escherichia coli* O111:B4 isolated by phenol-water extraction was obtained from Sigma Chemical Co. (St. Louis, Mo.). MPL from *Salmonella minnesota* R595 was prepared at Ribi Immunochemical Research, Inc. (Hamilton, Mont.), by using the extraction method of Galanos et al. (10). Characterization of this compound has been documented in previous papers (34, 39). Stock solutions of MPL and LPS were prepared by dissolving them in sterile water containing 0.2% triethylamine. The solutions were then clarified to slight opalescence after brief warming at 65°C and sonication. Poly(A)-poly(U) was a gift from the Institut Henri Beaufour (Les Ulis Cedex, France). Individual ampoules of the polynucleotide complex were dissolved in Click medium–10% FBS to give a dose of 10 μg when added in 0.025 ml. Murabutide was a gift from Louis Chedid (Institut Pasteur, Paris, France). Synthesis and characterization of this compound have been described elsewhere (6, 24, 25). Stock solutions of all adjuvants were stored at 4°C and appropriately diluted in Click medium–10% FBS before addition to the in vitro culture system. The final culture concentrations of the adjuvants used were as follows: LPS and MPL, 0.1 μg/ml; poly(A)-poly(U), 10 μg/ml; murabutide, 100 μg/ml.

MAb to IFN-γ. A monoclonal antibody (MAb) to IFN-γ (MAb-IFN-γ) was purchased from Lee Biomolecular Research Laboratories, Inc. (San Diego, Calif.) Thirteen thou-

sand units of lyophilized antibody was dissolved in 1.0 ml of sterile water, aliquoted, and frozen at −70°C until use. Individual aliquots were thawed and diluted 1/100 in Click medium–10% FBS prior to addition to cultures in 0.1 ml.

rMuIFN-γ. Recombinant murine IFN-γ (rMuIFN-γ) was purchased from Amgen Biologicals (Thousand Oaks, Calif.) and Genzyme Corp. (Boston, Mass.). The preparations were reconstituted and diluted according to technical instructions and frozen in aliquots at −70°C. Individual aliquots were thawed and diluted appropriately prior to addition to culture wells. Optimal doses for enhancement of antibody formation were found to be 1 U/ml for the Amgen preparation and 10 U/ml for the Genzyme preparation.

In vitro culture of spleen cells. Spleen cells were cultured by using a modified Mishell-Dutton system (30) as described previously (40). Briefly, spleen cells were washed and diluted in Click medium to 10^7 cells per ml. For cell separations, whole spleen cells were treated with Tris-ammonium chloride to remove erythrocytes. The remaining cells were washed three times before being counted and were diluted in Click medium. Cells were then added to plastic tissue culture plates (35 by 10 mm; two wells per experimental group). Cultures were incubated in the presence of antigen for 4 days in a 37°C humidified incubator containing 5% CO_2. On day 4, the cultures were harvested and plaque-forming cells (PFC) were measured.

T-cell enrichment of spleen cells. Whole spleen cells were incubated in 250-cm^2 tissue culture flasks (10 ml; 2.5×10^7 cells per ml in HBSS–5% FBS) for 2 h at 37°C–5% CO_2 to remove adherent cells. The nonadherent cells were poured off, the flasks were rinsed with 5 ml of HBSS, and the cells were centrifuged at 300 × *g* for 10 min. Following suspension in Click medium–10% FBS, the cells were counted and diluted to 10^8 cells per ml. The procedure of Julius et al. (19) was used for nylon wool purification of T cells. Scrubbed nylon fibers for column preparation were purchased from Cellular Products, Inc. (Buffalo, N.Y.) Three milliliters of the diluted nonadherent cells were added dropwise to a prepared nylon wool column and washed on with 1 ml of medium. Following incubation at 37°C–5% CO_2 for 45 min, effluent cells were collected by washing with 25 ml of medium, centrifuged, resuspended in Click medium–10% FBS, and counted. In tests for purity in the mitogenicity assay, a 30-fold stimulation was induced with concanavalin A, whereas LPS stimulated this population only 5-fold.

Activation and culture of T-cell-enriched population. A total of 10^7 cells per ml were incubated for 2 h at 37°C–5% CO_2 with the adjuvants at the doses given above. They were then centrifuged, washed three times with HBSS to remove any residual adjuvant, and suspended to the original volume with Click medium–10% FBS. For in vitro antibody production, 10^6 T-enriched cells were added to 9×10^6 whole spleen cells along with antigen and cultured to day 4. For generation of possible IFN-γ-containing supernatants, the adjuvant-activated T-enriched cells were incubated for 48 h at 37°C–5% CO_2. The cultures were harvested and centrifuged at 300 × *g* for 10 min, and the supernatants were collected and frozen until assayed for IFN-γ activity.

Hemolytic plaque assay. Antibody production was measured by using a modified hemolytic plaque assay which has been described in detail elsewhere (21, 40). Plastic tissue culture dishes (60 by 15 mm) were coated with 2 ml of poly-L-lysine (50 μg/ml; Sigma). After 15 min the plates were washed twice with phosphate-buffered saline (PBS; pH 7.2), and 2 ml of washed SRBC (4.5%) was added. Fifteen minutes later the plates were swirled and allowed to settle for another 15 min. They were then rinsed twice with PBS and covered with 1.5 ml of PBS. Spleen cells from pooled cultures were centrifuged, resuspended in PBS to two to three times the original volume, and added to the SRBC-coated tissue culture plates (three plates per experimental group), followed by the addition of 0.2 ml of guinea pig complement (Anderson Laboratories, Fort Worth, Tex.) diluted 1:2. The assay plates were incubated for 1 h at 37°C–5% CO_2. Plaques were then counted, and results are expressed as mean plaques per culture/viability.

Enzyme-linked immunosorbent assay to measure MuIFN-γ. A mouse IFN-γ enzyme-linked immunosorbent assay kit was purchased from Amgen. This kit utilized a first MAb specific for MuIFN-γ and a biotinylated second MAb to MuIFN-γ. An antibiotin-alkaline phosphatase conjugate was added as the detector complex and, after appropriate incubation and washing, a *p*-nitrophenylphosphate (PNPP) substrate was added. After addition of NaOH to stop the reaction, the A_{405} was measured. Units per milliliter was calculated for each sample, utilizing data from the standard curve run with each assay, giving standard points at 0, 5, 10, 20, and 40 U/ml.

Depletion of NK cells. Spleen cells were treated with anti-asialo GM1 (Wako Chemicals USA, Inc., Dallas, Tex.; final dilution, 1/50) for 1 h at 4°C. Following centrifugation at 300 × *g* for 10 min, the cells were suspended in low-toxicity rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada; final dilution, 1/12) and incubated for 1 h at 37°C–5% CO_2 in Click medium–10% FBS. Cedarlane cytotoxicity medium was used for all dilutions. After being centrifuged and washed, the cells were placed into in vitro culture.

Statistical analysis. Data were analyzed by a statistical program for a one-sample *t* test after the $\log_{10}$ ratio for each two groups being compared was determined. Statistical significance was assigned to groups showing a *P* value of <0.05.

RESULTS

Enhancement of antibody production by adjuvants. Microbial products representing three different classes of compounds have been well documented as adjuvants to the immune response. To verify this under our conditions, LPS, poly(A)-poly(U) complex, murabutide, and the nontoxic MPL were tested for their ability to enhance formation of antibody to SRBC in splenic cultures from BALB/c mice. The mean number of PFC from at least 11 experiments is illustrated in Fig. 1. MPL and LPS enhanced antibody formation to SRBC about sevenfold above that in controls receiving SRBC alone. Poly(A)-poly(U) and murabutide also were effective, but somewhat less so under these conditions, enhancing antibody formation about 3.5- and 2.5-fold, respectively.

Enhancement of antibody production by adjuvant-activated T cells. Our previous study with aging mice demonstrated that T lymphocytes are important mediators of the adjuvant action of a detoxified LPS, MPL (40). To determine whether a wild-type LPS and other adjuvants act in a like manner in young mice, we tested T cells that were treated with LPS, poly(A)-poly(U), and murabutide for their ability to elevate antibody formation on coculture. Control or adjuvant-stimulated T cells (10^6 cells) were added to unseparated spleen cells (9×10^6 cells) and incubated with antigen for 4 days. Results in Fig. 2 show the averaged data for 5 to 12 experiments. Each of the adjuvant-stimulated T-cell popula-

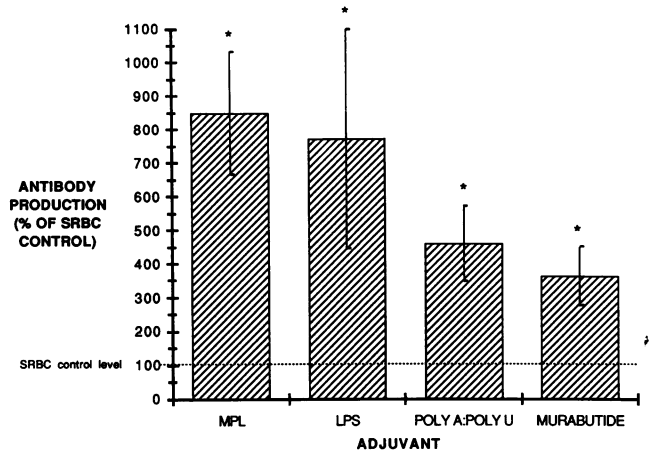

FIG. 1. Enhancement of antibody production by adjuvants. Medium or the adjuvants were added on day 0 to 10^7 BALB/c spleen cells and cultured in vitro with antigen. Data are means of at least 11 experiments ± standard errors of the means. *, $P < 0.001$. Control plaques per culture averaged 1,326.

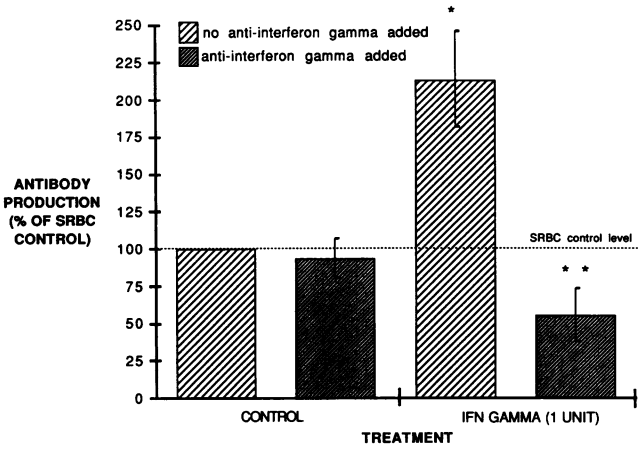

FIG. 3. IFN-γ increases production of antibody to SRBC. Recombinant IFN-γ (Amgen; 1.0 U) was added to 10^7 BALB/c spleen cells on day 0. Data are means ± standard errors of the means of at least four experiments. *, $P < 0.005$ versus control; **, $P < 0.0005$ versus IFN-γ treatment. Control plaques per culture averaged 968.

tions on coculture increased the number of cells secreting antibody to double or triple the control value. However, the murabutide-activated T-cell enhancement was not statistically significant because of interexperimental variability.

Enhancement of antibody production by rMuIFN-γ. IFN-γ has been shown to both enhance and suppress antibody formation (18, 37), depending on experimental conditions, and also to be integrally involved in generation of a T-cell-dependent antibody response (13, 27). Consequently, a wide range of rMuIFN-γ doses were tested without the adjuvants in preliminary experiments for their ability to affect in vitro antibody production in splenic cultures from young mice. One U/ml (Amgen) or 10 U/ml (Genzyme) was found to give optimal enhancement (data not shown). The data from four to eight experiments demonstrate that 1 U of rMuIFN-γ (Amgen) per ml doubled antibody formation over that in controls (Fig. 3). This enhancement was totally blocked upon addition of 13 neutralizing units of MAb-IFN-γ to the wells at the initiation of culture. Addition of the antibody to control wells had no effect. The results presented in Fig. 4 demonstrate that although IFN-γ caused maximal enhancement when added on day 0, significant enhancement was also seen when it was added on day 1, 2, or 3.

Blockage of adjuvant-induced enhancement by MAb-IFN-γ. Enhancement of antibody production by MPL was previously shown to be blocked by a MAb-IFN-γ (40). Consequently, MAb-IFN-γ was tested for its ability to similarly inhibit the enhancement by the wild-type LPS, poly(A)-poly(U), and murabutide. Accordingly, 13 neutralizing units of MAb-IFN-γ was added at culture initiation to spleen cells which had been activated by the adjuvants and to control cultures.

The results of at least five experiments show that MAb-IFN-γ significantly decreased the enhancement induced by

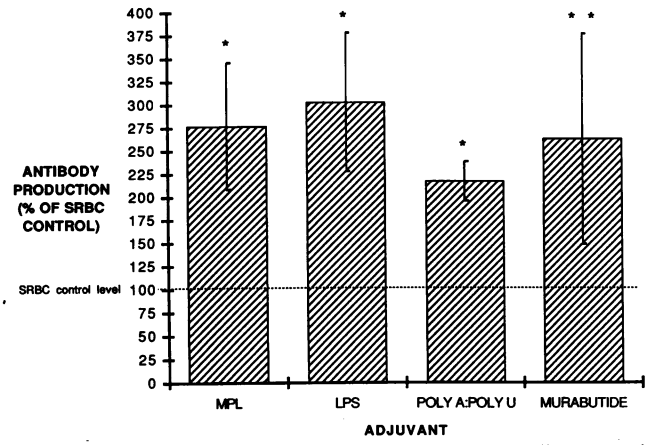

FIG. 2. Enhancement of antibody production by adjuvant-activated T cells. T cells were purified by a 2-h adherence to plastic followed by passage over nylon wool; they were then incubated with adjuvant for 2 h. A total of 10^6 control or adjuvant-treated T cells were added to 9×10^6 whole spleen cells along with SRBC and cultured in vitro to day 4. Data are means of at least five experiments ± standard errors of the means. *, $P < 0.005$; **, $P < 0.09$. Control plaques per culture averaged 1,647.

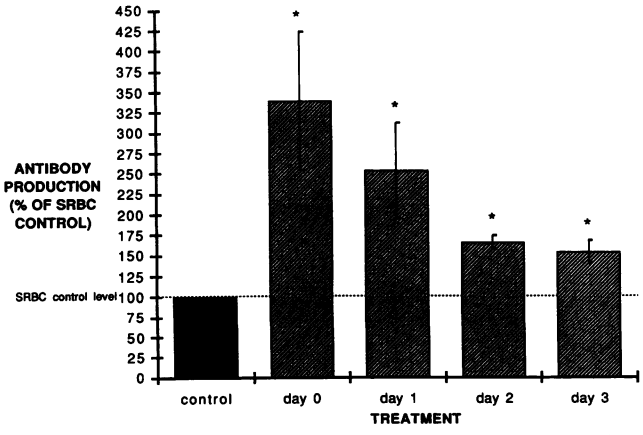

FIG. 4. Kinetics of IFN-γ addition to spleen cell cultures from young adult BALB/c mice in vitro. Recombinant IFN-γ (Genzyme; 10.0 U) was added to 10^7 BALB/c spleen cells on day 0, 1, 2, or 3. Data are means ± standard errors of the means of four experiments. *, $P < 0.05$. Control plaques per culture averaged 1,769.

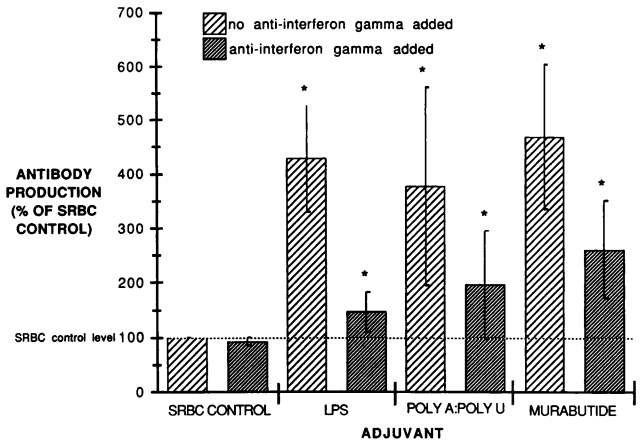

FIG. 5. Blocking of adjuvant enhancement by anti-IFN-γ antibody. Medium or adjuvants were added to BALB/c spleen cells on day 0 along with SRBC. Data are means of at least five experiments ± standard errors of the means. *, $P < 0.05$ versus appropriate control. Control plaques per culture averaged 1,320.

LPS (66%), poly(A)-poly(U) (48%), and murabutide (44%) (Fig. 5). Addition of MAb to control cultures did not significantly affect antibody production.

Augmentation of IFN-γ release by treatment with adjuvant. Since IFN-γ was implicated as a mediator released from adjuvant-activated T cells and 13 U of MAb-IFN-γ blocked approximately half of the adjuvant-induced enhancement, we tested whether adjuvant-activated T cells released increased levels of IFN-γ compared with control T cells. Accordingly, T cells enriched by removal of adherent cells and passage over nylon wool were activated for 2 h with the adjuvants, washed, and cultured for 48 h. The IFN-γ titers in the resultant supernatant fluids from four experiments are shown in Fig. 6. MPL, LPS, and murabutide induced significant increases in IFN-γ release by T cells, which averaged about threefold higher than that in controls, while poly(A)-poly(U) doubled IFN-γ levels.

NK cells and IFN-γ. NK cells, as well as T lymphocytes,

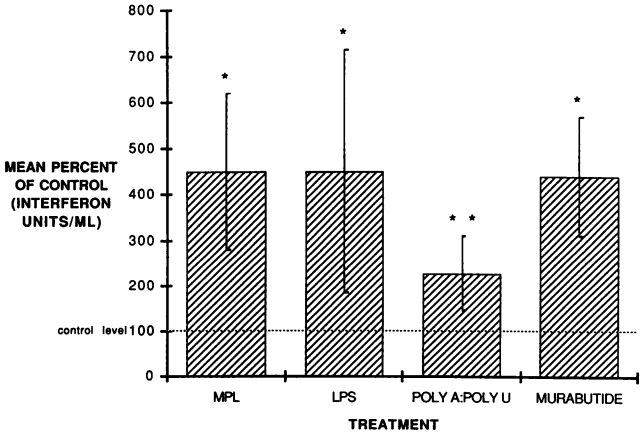

FIG. 6. IFN-γ release is augmented by 2-h adjuvant activation of enriched T cells. Enriched T cells (10^7) were activated for 2 h with adjuvants, washed three times, and suspended in medium. After 48 h, supernatants were collected and frozen until an enzyme-linked immunosorbent assay was performed. Data are means ± standard errors of the means of four experiments. *, $P < 0.05$; **, $P < 0.12$. Control IFN units per milliliter averaged 1.10.

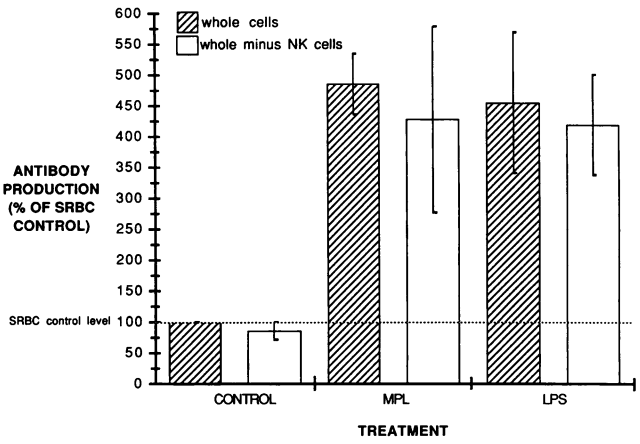

FIG. 7. NK cells do not mediate the adjuvant action of MPL or LPS. Data are means of two experiments ± standard errors of the means. Control plaques per culture averaged 419.

have been shown to release IFN-γ (7). NK cell-depleted spleen cell populations were tested for their responsiveness to MPL and LPS. There was no loss in the adjuvanticity of MPL or LPS in two experiments when spleen cells were treated with anti-asialo GM1 to remove NK cells (Fig. 7). This antibody removed essentially all NK cell activity when tested in our laboratory (data not shown).

DISCUSSION

The three adjuvants under study in this investigation are from diverse sources and are structurally dissimilar. Yet it appears that IFN-γ release is integrally involved in their stimulatory action. Although each most likely has a different receptor, the data suggest the interesting possibility that the transmembrane signals activated by each adjuvant work through the same intracellular pathway and G-protein signal transducer leading to IFN-γ release.

The results obtained in this study implicate IFN-γ-producing T cells (T_{h1}) as initial target cells of the adjuvants. T_c cells were ruled out as the source of IFN-γ in previous work with MPL (40), in which enhancement remained following treatment with monoclonal anti-Lyt2.2 antibody plus complement. The IFN-γ released presumably increases Ia antigen (38) and/or interleukin-1 (IL-1) (3) release from macrophages, leading to increased antibody production. Acceptance of this hypothesis is tempered with the knowledge that these immunomodulators are known to affect directly the release of IL-1 from macrophages (31, 33; unpublished observations). In addition, several investigators have reported IL-1-induced release of IL-2 as obligatory to IFN-γ release (8, 9, 23, 41), leading to the rationale that the initial target cell should be the macrophage. However, T_{h1} clones are known to synthesize IL-2 and IFN-γ but lack receptors for IL-1 and do not respond to IL-1 (12, 20). Since all three adjuvants increase IFN-γ release, the hypothesis favored is that IFN-γ is the initiating cytokine of the resultant enhancement of antibody formation. It should also be emphasized that following a mitogenic stimulus, IFN-γ mRNA production has been shown to be biphasic, with accumulation peaking at 60 min and 20 h (11). The initial mRNA peak for IFN-γ appeared simultaneously with that of IL-2 mRNA, suggesting that the early IFN-γ release is not dependent on IL-2 (11). The late-appearing IFN-γ is decreased by exogenous IL-2 and antibody specific for the IL-2

receptor (Tac) and thus depends on IL-2–IL-2 receptor interaction (42).

These observations offer an explanation for the recent results of Tomai and Johnson (40). When MPL was given in vivo 2 h prior to sacrifice, followed by transfer of isolated cell populations to a Mishell-Dutton system, macrophages were found capable of transferring MPL-induced enhancement. However, when MPL was added to a totally in vitro system, only T cells were found capable of transferring the adjuvant action. The explanation for this dichotomy may lie in the probability that the 2-h interval following in vivo injection was sufficient time for the adjuvant to activate T cells to release IFN-γ. The IFN-γ then could instill in macrophages the capability for continuation of the cascade leading to increased numbers of PFC. The rapid induction of IL-1 by IFN-γ also may account for the inability of anti-IFN-γ antibody to block completely the enhancing action, despite the use of high levels of antibody (data not shown).

T cells have been implicated previously as being affected by all three adjuvants used in this study. Thus, Allison and Davies (2), McGhee et al. (29), Nakano et al. (32), and Vogel et al. (43) all have reported the involvement of T cells in the adjuvant action of LPS. In addition, Baker et al. (4) and Tomai and Johnson (40) have identified a T cell as the target of the detoxified endotoxin, MPL. Bick and Johnson (5) reported secretion of a helper factor from T lymphocytes following poly(A)-poly(U) stimulation which increased antibody production. This followed earlier studies showing that poly(A)-poly(U) amplifies thymus cell functions (16). More recently, Hovanessian et al. (15) reported that poly(A)-poly(U) stimulation resulted in increased p67K kinase activity in mice and humans, which has been shown to be a marker for the presence of IFN-γ. Murabutide has also been shown to act on T cells (24). These studies coupled with the demonstration by Lei and Morrison (26) of LPS-binding receptors on murine splenocytes, including T cells, offer evidence for an initial adjuvant signal bestowed on T cells. Although NK cells are known to possess immunoregulatory capability (1) and to secrete IFN-γ, any role for NK cells is unlikely since enhancement by both MPL and LPS remained after NK cells were removed by in vitro treatment with anti-asialo GM1 antibody (Fig. 7).

Despite reports that IFN-γ suppresses the antibody response (17, 18) and is required for the activity of natural suppressor cells (14), other studies have demonstrated a positive or perhaps even obligatory requirement for IFN-γ. Thus, Sonnenfeld et al. (37) and Shalaby et al. (35) found that IFN-γ enhanced the antibody responses when added exogenously. It was suggested (37) that the antibody enhancement was mediated by T lymphocytes while the observed immunosuppression was mediated by B lymphocytes. Several investigators (13, 27) have shown that IFN-γ is involved in, if not required for, the normal generation of antibody by B cells. We could not conclude this from our experiments, however, even though the MAb-IFN-γ used originated from the same hybridoma cell line as that used by Helman and Wallace (13). When we added the antibody in vitro on day 0 to spleen cell cultures with SRBC (without adjuvant) in 43 experiments, the mean percentage ± standard error of the mean relative to the control without antibody was 93 ± 9. This dose (13 U) of MAb-IFN-γ was sufficient to significantly decrease adjuvant-induced enhancement of antibody production (Fig. 5). An explanation of the different results may be that high doses of antigen induce increased amounts of IFN-γ, since Helman and Wallace (13) used approximately 10-fold more SRBC than was used in our experiments. The experiments by Langford et al. (22), which also showed blocking of the antibody response to SRBC with MAb-IFN-γ, used both a high SRBC dose (10^8 cells) and the intravenous route, which is generally more effective in generating antibody than the intraperitoneal route. Since we did not stimulate with antigen at a high level in our SRBC control group, it is probable that IFN-γ was not being produced optimally and could then be induced by the adjuvants, resulting in increased numbers of PFC. This is supported by the oft-noted evidence that adjuvant activity is most obvious in models in which antibody synthesis is below normal and raises the question whether the IFN-γ levels are a key controlling variable in those situations.

While differing experimental conditions may explain in part the opposing suppression and enhancement induced by IFN-γ, no general concept has emerged as to the immunoregulatory mechanism of this cytokine. Though the adjuvants seem to act via IFN-γ released by T cells very early in the response (1 to 2 h), IFN-γ also significantly increased antibody production when added late to cultures (Fig. 4). In support of this, Leibson et al. (28) added IL-X, which was later identified as IFN-γ (27), to purified B cells with IL-1 and P388D1 supernatants on days 0, 1, and 2 of culture. Peak response was seen with IL-X (IFN-γ) addition on day 1, but a significant increase over cultures with only IL-1 and P388D1 supernatants was seen with IL-X added on days 0 and 2. A possible explanation of this observation comes from the results of Sidman et al. (36), which suggest that IFN-γ may be involved in the maturation of B cells to active antibody production, which occurs late in the response. Thus, the observed biphasic release of IFN-γ reported by Grabstein et al. (11) may have the following explanation. Early induction of IFN-γ by a mitogen, adjuvant, or antigen results in macrophage activation and increased levels of Ia antigen and IL-1. IL-1 increases IL-2 receptor expression and IL-2 release, inducing additional IFN-γ, which in turn aids in the maturation of B cells to immunoglobulin secretion.

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LITERATURE CITED

1. **Abruzzo, L. V., and D. A. Rowley.** 1983. Homeostasis of the antibody response: immunoregulation by NK cells. Science **222**:581–585.
2. **Allison, A. C., and A. J. S. Davies.** 1971. Requirement of thymus-dependent lymphocytes for potentiation by adjuvants of antibody formation. Nature (London) **233**:330–332.
3. **Arenzana-Seisdedos, F., J. L. Virelizier, and W. Fiers.** 1985. Interferons as macrophage-activating factors. III. Preferential effects of interferon-γ on the interleukin-1 secretory potential of fresh or aged human monocytes. J. Immunol. **134**:2444–2448.
4. **Baker, P. J., J. R. Hiernaux, M. B. Fauntleroy, B. Prescott, J. L. Cantrell, and J. A. Rudbach.** 1988. Inactivation of suppressor T-cell activity by nontoxic monophosphoryl lipid A. Infect. Immun. **56**:1076–1083.
5. **Bick, P. H., and A. G. Johnson.** 1977. Poly A:U-induced secretion of T-lymphocyte helper factors. Scand. J. Immunol. **6**:1133–1144.
6. **Chedid, L., M. Parant, F. Audibert, G. Riveau, F. Parant, E. Lederer, J. Choay, and P. Lefrancier.** 1982. Biological activity of a new synthetic muramyl peptide adjuvant devoid of pyrogenicity. Infect. Immun. **35**:417–424.
7. **Djeu, J. Y., N. Stocks, K. Zoon, G. J. Stanton, T. Timonen, and R. B. Herberman.** 1982. Positive self regulation of cytotoxicity in human natural killer cells by production of interferon upon

exposure to influenza and herpes viruses. J. Exp. Med. **156:** 1222–1234.
8. **Epstein, L. B., M. J. Cline, and T. C. Merigan.** 1971. PPD-stimulated interferon: *in vitro* macrophage-lymphocyte interaction in the production of a mediator of cellular immunity. Cell. Immunol. **2:**602–613.
9. **Farrar, W. L., H. M. Johnson, and J. J. Farrar.** 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin 2. J. Immunol. **126:**1120–1125.
10. **Galanos, C., O. Luderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9:**245–249.
11. **Grabstein, K., S. Dower, S. Gillis, D. Urdal, and A. Larsen.** 1986. Expression of interleukin 2, interferon-γ and the IL-2 receptor by human peripheral blood lymphocytes. J. Immunol. **136:**4503–4508.
12. **Greenbaum, L. A., J. B. Horowitz, A. Woods, T. Pasqualini, E. Reich, and K. Bottomly.** 1988. Autocrine growth of $CD4^+$ T cells. Differential effects of IL-1 on helper and inflammatory T cells. J. Immunol. **140:**1555–1560.
13. **Helman, S. W., and J. H. Wallace.** 1989. Differential regulation of the immune response to SRBC by monoclonal antibodies to interferon-γ. Proc. Soc. Exp. Biol. Med. **191:**55–59.
14. **Holda, J. H., T. Maier, and H. N. Claman.** 1988. Evidence that IFN γ is responsible for natural suppressor activity in GVHD spleen and normal bone marrow. Transplantation **45:**772–777.
15. **Hovanessian, A. G., Y. Riviere, L. Montagnier, M. Michelson, J. Lacour, and F. Lacour.** 1982. Enhancement of interferon-mediated protein kinase in mouse and human plasma in response to treatment with poly A:poly U. J. Interferon Res. **2:**209–215.
16. **Johnson, A. G.** Modulation of the immune system by synthetic polynucleotides. 1979. Springer Semin. Immunopathol. **2:**149–168.
17. **Johnson, H. M., and S. Baron.** 1976. The nature of the suppressive effect of interferon and interferon inducers on the *in vitro* immune response. Cell. Immunol. **25:**106–115.
18. **Johnson, H. M., and B. A. Torres.** 1983. Recombinant mouse interferon-γ regulation of antibody production. Infect. Immun. **41:**546–548.
19. **Julius, M., E. Simpson, and L. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. **3:**645–649.
20. **Kaye, J., S. Gillis, S. B. Mizel, E. M. Shevach, T. R. Malek, C. A. Dinarello, L. B. Lachman, and C. A. Janeway, Jr.** 1984. Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin 1 is required for the expression of receptors for interleukin 2. J. Immunol. **133:**1339–1345.
21. **Kennedy, J., and M. Axelrad.** 1971. An improved assay for hemolytic plaque-forming cells. Immunology **20:**253–261.
22. **Langford, M. P., D. A. Weigent, T. S. Chan, H. M. Johnson, and G. J. Stanton.** 1987. Antibodies to the carboxyl terminus of mouse interferon-γ neutralize its immunoregulatory and antiviral activities. J. Interferon Res. **7:**95–101.
23. **Le, J., J. X. Lin, D. Henriksen-DeStefano, and J. Vilcek.** 1986. Bacterial lipopolysaccharide-induced interferon-gamma production: roles of interleukin 1 and interleukin 2. J. Immunol. **136:**4525–4530.
24. **Leclerc, C., and F. R. Vogel.** 1986. Synthetic immunomodulators and synthetic vaccines. Crit. Rev. Ther. Drug Carrier Syst. **2:**353–406.
25. **Lefrancier, P., M. Derrien, X. Jamet, J. Choay, E. Lederer, F. Audibert, M. Parant, F. Parant, and L. Chedid.** 1982. Apyrogenic, adjuvant-active N-acetyl-muramyl dipeptides. J. Med. Chem. **25:**87–90.
26. **Lei, M.-G., and D. C. Morrison.** 1988. Specific endotoxic lipopolysaccharide-binding proteins on murine splenocytes. I. Detection of lipopolysaccharide-binding sites on splenocytes and splenocyte subpopulations. J. Immunol. **141:**996–1005.
27. **Leibson, H. J., M. Gefter, A. Zlotnik, P. Marrack, and J. W. Kappler.** 1984. Role of γ-interferon in antibody-producing responses. Nature (London) **309:**799–801.
28. **Leibson, H. J., P. Marrack, and J. Kappler.** 1982. B cell helper factors. II. Synergy among three helper factors in the response of T cell- and macrophage-depleted B cells. J. Immunol. **129:** 1398–1402.
29. **McGhee, J. R., J. J. Farrar, S. M. Michalek, S. E. Mergenhagen, and D. L. Rosenstreich.** 1979. Cellular requirements for lipopolysaccharide adjuvanticity. A role for both T lymphocytes and macrophages for *in vitro* responses to particulate antigens. J. Exp. Med. **149:**793–807.
30. **Mishell, R., and R. Dutton.** 1967. Immunization of dissociated spleen cell cultures in normal mice. J. Exp. Med. **126:**423–442.
31. **Morrison, D. C., and J. L. Ryan.** 1979. Bacterial endotoxins and host immune responses. Adv. Immunol. **28:**293–451.
32. **Nakano, M., T. Uchiyama, and K. Saito.** 1973. Adjuvant effect of endotoxin: antibody response to sheep erythrocytes in mice after transfer of syngeneic lymphoid cells treated with bacterial lipopolysaccharide *in vitro*. J. Immunol. **110:**408–413.
33. **Oppenheim, J. J., A. Togawa, L. Chedid, and S. Mizel.** 1980. Components of mycobacteria and muramyl dipeptide with adjuvant activity induce lymphocyte activating factor. Cell. Immunol. **50:**71–81.
34. **Qureshi, N., and K. Takayama.** 1982. Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of *Salmonella minnesota*. J. Biol. Chem. **257:**11808–11815.
35. **Shalaby, M. R., P. K. Weck, E. Rinderknecht, R. N. Harkins, J. W. Frane, and M. J. Ross.** 1984. Effects of bacteria-produced human alpha, beta and gamma interferons on *in vitro* immune functions. Cell. Immunol. **84:**380–392.
36. **Sidman, C. L., J. D. Marshall, L. D. Shulz, P. W. Gray, and H. M. Johnson.** 1984. γ-Interferon is one of several direct B cell maturing lymphokines. Nature (London) **309:**801–803.
37. **Sonnenfeld, G., A. D. Mandel, and T. C. Merigan.** 1978. Time and dosage dependence of immunoenhancement by murine type II interferon preparations. Cell. Immunol. **40:**285–293.
38. **Steeg, P. S., R. N. Moore, H. M. Johnson, and J. J. Oppenheim.** 1982. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. J. Exp. Med. **156:**1780–1793.
39. **Takayama, K., E. Ribi, and J. L. Cantrell.** 1981. Isolation of a nontoxic lipid A fraction containing tumor regression activity. Cancer Res. **41:**2654–2657.
40. **Tomai, M. A., and A. G. Johnson.** 1989. Gamma interferon mediates the adjuvant action of a detoxified endotoxin. J. Biol. Response Modif. **8:**643–661.
41. **Torres, B. A., W. L. Farrar, and H. M. Johnson.** 1982. Interleukin 2 regulates immune interferon (IFN gamma) production by normal and suppressor cell cultures. J. Immunol. **128:** 2217–2219.
42. **Vilcek, J., D. Henriksen-DeStefano, D. Siegel, A. Klion, R. J. Robb, and J. Le.** 1985. Regulation of IFN γ induction in human peripheral blood cells by exogenous and endogenously produced interleukin 2. J. Immunol. **135:**1851–1856.
43. **Vogel, S. N., M. L. Hilfiker, and M. J. Caulfield.** 1983. Endotoxin-induced T lymphocyte proliferation. J. Immunol. **130:** 1774–1779.