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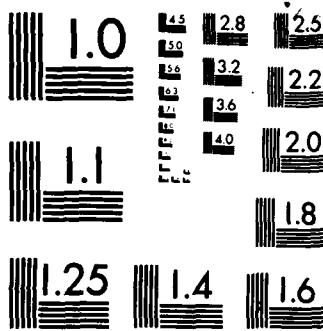
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  
In addition to antiviral activity, the three antigenically distinct species ( $\alpha, \beta, \gamma$ ) of interferon (IFN) exhibit multiple biological activities, some of which suggest, possible immunomodulatory roles for these molecules in either the generation and/or expression of immunity. The ultimate objective of our research is to determine what actions each IFN may mediate during cell-mediated immune responses. Our immediate goal is to study the effects of these antiviral molecules on macrophage functions, both in vivo and in vitro murine systems. Interest in IFN actions on macrophage function(s) is based on the

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facts that this cell is an intergral element in cellular immunity, often executing effector functions, as well as the reports that various preparations possessing antiviral activity mediated by IFN enhanced the expression of many parameters associated with activated macrophages. To establish whether a particular effect is due to an IFN, either pure murine IFNs (MuIFN) or their respective monospecific or monoclonal neutralizing antibodies are required. Our efforts over the past year have centered on the characterization of the lymphokine MuIFN $\gamma$  and the generation of neutralizing monoclonal anti-MuIFN $\gamma$  which is being used as a probe in vitro and eventually will be used in vivo, to elucidate MuIFN $\gamma$  actions on macrophages and the role of this T-cell product in cellular immunity. The following is a list of our accomplishments.

1. The successful immunization of rats with a partially purified IFN $\gamma$  produced by the closely related murine species.
2. The generation of transspecies hybridomas through the fusion of rat immune B-cells to mouse myeloma (P3U-1) cells. Screening the supernatants of hundreds of wells containing hybridomas from multiple fusion efforts, revealed that the medium from a single well possessed the ability to neutralize the antiviral activity of MuIFN $\gamma$ . From this well, a hybridoma secreting a rat anti-MuIFN $\gamma$  monoclonal antibody (MAB) was cloned.
3. The hybridoma producing rat anti-MuIFN $\gamma$  MAB has proven stable and is being propagated both in vitro and as well as in vivo in nude mice as ascites. One ml of crude culture supernatant can neutralize 2000 units of MuIFN $\gamma$  antiviral activity, whereas, the ascitic fluids are 1,000 times more potent.
4. Characterization studies as to the potential of the MAB to neutralize various biological activities of lymphokine preparations thought to be mediated by MuIFN $\gamma$ , have been initiated. These studies have so far established that the MAB neutralizes not only the antiviral and anticellular (growth inhibition) activities of crude lymphokine preparations but also, the ability of these preparations to activate the tumoricidal action of macrophages. This latter observation indicates that MuIFN $\gamma$  is also macrophage activation factor (MAF).

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Generation of Rat anti-MuIFN $\gamma$  Monoclonal Antibody (MAb) and  
Its Neutralizing Activities

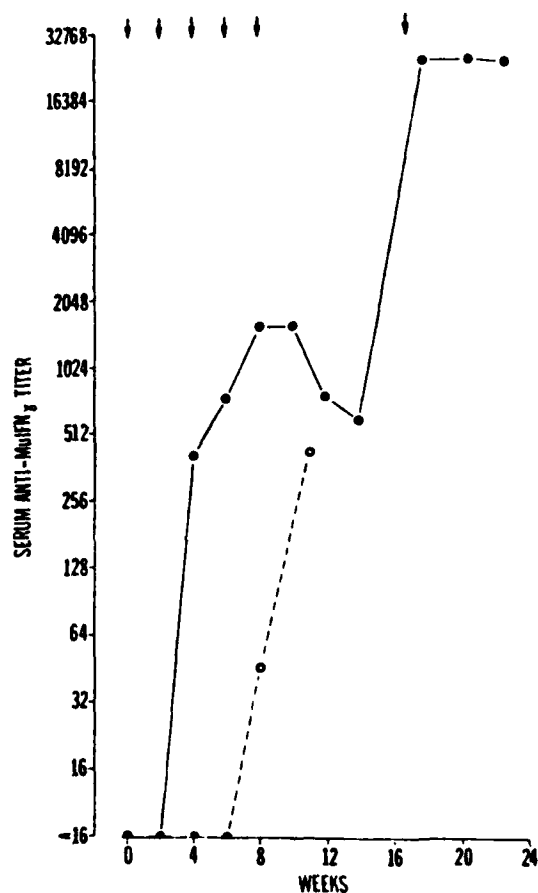
1. Immunization of rats with MuIFN $\gamma$ . High-titered preparations (1000 units/ml) of MuIFN $\gamma$  were produced *in vitro* by stimulating spleen cells ( $10^7$  cells/ml), obtained from AB6F $_1$  mice challenged intravenously 6 days earlier with an immunizing *Listeria* dose ( $4 \times 10^3$  organisms), with the polyclonal T-cell mitogen phytohemagglutinin (PHA). The 24 hr culture supernatants were concentrated 10-fold by Amicon PM-10 ultrafiltration. A MuIFN $\gamma$  fraction (60% total activity) was isolated, purified, and further concentrated (60,000 units/ml) by means of concanavalin A (Con-A) lectin affinity chromatography. This major fraction of MuIFN $\gamma$  which bound to the lectin and was specifically eluted with the counterligand  $\alpha$ -methyl-D-mannoside was purified 50-fold over the starting preparation to a specific activity of  $5 \times 10^5$  units/mg of protein. This partially purified Con A-bound MuIFN $\gamma$  served as the immunogen in the following studies.

Initially, rats (DA strain) were injected with a total of 2 ml of MuIFN $\gamma$ -adjuvant emulsion at multiple subcutaneous sites on a biweekly basis. The first two biweekly immunizations were done with Freund's complete adjuvant, which was emulsified with an equal volume of the Con A-bound MuIFN $\gamma$  (32,000 units/ml). All subsequent immunizations were carried out with the same MuIFN $\gamma$  preparation emulsified with Freund's incomplete adjuvant. Prior to each immunization, animals were bled, sera collected and heat-inactivated for 30 min. Sera were stored at  $-20^\circ\text{C}$ , until assayed for anti-MuIFN $\gamma$  neutralizing activity. The anti-MuIFN $\gamma$  neutralizing titer of an antibody preparation is defined as the reciprocal of the highest dilution of the antibody which when reacted with an equal volume of MuIFN $\gamma$  (final concentration of 10 antiviral units/ml) neutralizes 50% of the antiviral activity as judged by the development of vesicular stomatitis virus cytopathic effect on L929B cells.

The appearance of titers of serum neutralizing anti-MuIFN $\gamma$  antibodies during the course of immunization of both a typical rat as well as a rabbit are presented in Fig. 1. While both the rat and rabbit were immunized according to the same schedule with the same doses of antigen, the rat responded with neutralizing anti-MuIFN $\gamma$  later and with lower levels of neutralizing activity than the rabbit. The specificities of both polyclonal anti-MuIFN $\gamma$  neutralizing sera were examined in neutralization assays against a variety of IFN preparations. Neither anti-MuIFN $\gamma$  sera neutralized the antiviral activities of MuIFN $\alpha/\beta$  or HuIFN $\gamma$  preparations. The rabbit and rat anti-MuIFN $\gamma$  sera, which were raised against the Con A-bound MuIFN $\gamma$ , neutralized the total antiviral activity of the crude unfractionated MuIFN $\gamma$  from which the immunogen was isolated. This indicates that these antisera also neutralize the antiviral activity of the MuIFN $\gamma$  component present in the unfractionated preparation, which does not bind to immobilized Con A (Con A unbound MuIFN $\gamma$ ). The rat anti-MuIFN $\gamma$  did not cross-react to neutralize rat IFN $\gamma$  whereas, the rabbit anti-MuIFN $\gamma$  did.

Fig. 1

Immunization Schedules and Appearance of Rabbit (●) and Rat (○) serum Anti-MuIFN $\gamma$  Neutralizing Activities. Time of Immunization (↓).



This indicates that the rabbit recognized epitopes common to both murine and rat MuIFN $\gamma$  and based on the specificity of the rat immune serum, it can be concluded that the rat recognizes an antigenic determinant(s) unique to the MuIFN $\gamma$  molecule.

## 2. Derivation of a Hybridoma secreting Rat anti-MuIFN $\gamma$ Monoclonal Antibody.

The successful immunization of rats with MuIFN $\gamma$  enabled us to fuse rat immune spleen B cells with mouse myeloma cells (P3U-1) in an attempt to produce a hybridoma which secretes rat neutralizing anti-MuIFN $\gamma$  antibody. The following is a brief description of the procedures used to obtain a hybridoma producing monoclonal

anti-MuIFN $\gamma$ . Once rats were found to possess high-titered serum anti-MuIFN $\gamma$  activity, they were again immunized intravenously. Four days later, the rat spleen cells were mixed at a 5:1 ratio with mouse P3U-1 myeloma and hybridomas were generated in the presence of polyethylene glycol 1450. Cells were suspended in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS) and seeded into 16mm wells. The following day, all wells received an additional equal volume of medium containing 2x hypoxanthine, aminoterin and thymidine (H.A.T.). This medium was removed one week later and the cultures replenished with medium containing only H.T. After 7 to 10 days, the hybridoma supernatants were screened for antibodies capable of neutralizing the antiviral activity of MuIFN $\gamma$ . Following three fusion and screening efforts, the supernatant of a single well from hundreds of wells containing the fusion products from the fourth immune rat possessed neutralizing activity for MuIFN $\gamma$ . Positive hybridomas from this well were subcloned twice by limiting dilution in microtiter plates with irradiated thymocyte feeder layers in DMEM containing 20% FBS. The cloned hybridoma (R4-6A2) was expanded and the isotype of monoclonal antibody (MAB) produced by this clone was determined to be in the rat IgG<sub>1</sub> subclass. Culture supernatants were made to 45% saturation and the precipitate (IgG) was resuspended in phosphate buffered saline (PBS) pH7.4 to 1/30 original volume, dialyzed exhaustively in PBS and then sterile filtered.

The specificity of the neutralizing activity of the concentrated MAB was tested against a variety of MuIFN $\gamma$  preparations. The results in Table 1 reveal that irregardless of the source of MuIFN $\gamma$ , the antiviral activity of each MuIFN $\gamma$  preparation was neutralized to an approximately similar degree as the immunogen (PHA-induced MuIFN $\gamma$ , Con A-bound fraction). In contrast, the antiviral activities of MuIFN $\alpha$ +MuIFN $\beta$  preparations, human IFN $\gamma$  and rat IFN $\gamma$  were not neutralized by the MAB. The observation that the antiviral activities of glycosylated and non-glycosylated (recombinant MuIFN $\gamma$  produced by *E. coli*) MuIFN $\gamma$  were neutralized equally, indicates that the epitope recognized by the MAB resides within the polypeptide structure of the MuIFN $\gamma$  molecule.

Further characterization studies of the MAB has established that the amount of MAB required to neutralize MuIFN $\gamma$  is directly proportional to the titer of antiviral activity (antigenic mass). In Figure 2, a dose-response curve for the MAB neutralization of homologous (murine) and heterologous (rat) antiviral activities reveals a linear relationship exists between the quantity of MAB required to neutralize a defined amount of antiviral activity (antigenic mass). Since the rat fibroblast cells are 2-times less sensitive than homologous murine cells to MuIFN $\gamma$  antiviral activity, twice as much MAB was required to abate an equivalent amount of MuIFN $\gamma$  antiviral activity on rat cells than a similar amount of antiviral activity on homologous murine cells.

TABLE 1

4.

Specificity of Rat Monoclonal Anti-MuIFN $\gamma$ 

| Interferon           | Production Mode              |                               | Fraction                   | Neutralization titer <sup>a</sup><br>for antiviral activity |
|----------------------|------------------------------|-------------------------------|----------------------------|---|
|                      | System                       | Induction                     |                            |   |
| MuIFN $\gamma$       | Spleen cells                 | Con-A <sup>b</sup>            | -                          | 14,000  |
| MuIFN $\gamma$       | Spleen cells                 | PHA <sup>c</sup>              | -                          | 13,000  |
| MuIFN $\gamma$       | Spleen cells                 | PHA <sup>d</sup>              | Con-A-Bound <sup>d</sup>   | 13,000  |
| MuIFN $\gamma$       | Spleen cells                 | PHA <sup>d</sup>              | Con-A-Unbound <sup>d</sup> | 13,000  |
| MuIFN $\gamma$       | recombinant DNA <sup>e</sup> | -                             | -                          | 13,000  |
| MuIFN $\gamma$       | Serum                        | BCG/OT <sup>f</sup>           | -                          | 13,000  |
| MuIFN $\gamma$       | MLR                          | Allogeneic cells <sup>g</sup> | -                          | 26,000  |
| MuIFN $\alpha+\beta$ | C-243                        | virus                         | -                          | <20   |
| Rat IFN $\gamma$     | Spleen cells                 | PHA <sup>i</sup>              | -                          | <20   |
| HuIFN $\gamma$       | Blood leucocytes             | PHA <sup>j</sup>              | -                          | <20   |

Neutralization titer is defined as the reciprocal of the highest dilution of antibody which when mixed with an equal volume of IFN $\gamma$  (Final IFN $\gamma$  concentration 10 U/ml) neutralizes 50% of the antiviral activity as judged by the development of viral cytopathic effect.

Spleen cells from Ax(C57BL/6F<sub>1</sub>) mice injected 6 days earlier with  $2 \times 10^3$  Listeria monocytogenes were suspended at  $10^7$  cells/ml in RPMI 1640 with 10  $\mu$ g/ml gentamycin (Schering) and stimulated in vitro with 2  $\mu$ g/ml Con-A (Type IV; Sigma) for 24 hrs.

Same as in b, except spleen cells were stimulated with 5  $\mu$ g/ml PHA (Burroughs-Wellcome).

A PHA-induced MuIFN $\gamma$  was fractionated by Con-A affinity chromatography into two fractions: one fraction which specifically bound to the lectin column (Con-A-bound) and a minor fraction which did not bind (Con-A-unbound).

Highly purified IFN $\gamma$  ( $>10^7$  U/mg) produced by cloned MuIFN $\gamma$  gene transfected to E. coli and kindly supplied by Genentech, Inc.

Ax(C57BL/6F<sub>1</sub>) mice were injected with  $5 \times 10^6$  Mycobacterium bovis (BCG) and 21 days later inoculated with old tuberculin (O.T.; 10 mg total protein; Jensen-Salisbury Labs). Serum was collected 2 hrs. after injection of old tuberculin.

For mixed-lymphocyte reaction (MLR)-induced IFN $\gamma$ , responder C57BL/6xDBA/2 F<sub>1</sub> mice were injected in both hind footpads 9-21 days earlier with  $2 \times 10^6$  allogeneic (H-2<sup>a</sup>) SA-1 tumor cells. Responder spleen cells were mixed with an equal number ( $4-5 \times 10^6$ ) of gamma-irradiated (1000r) stimulator spleen cells from A strain mice and supernatants were collected 72 hours later.

Pure IFN $\alpha+\beta$  was produced by Newcastle disease virus-induced mouse C-243 cells and purchased from Enzo Biochem.

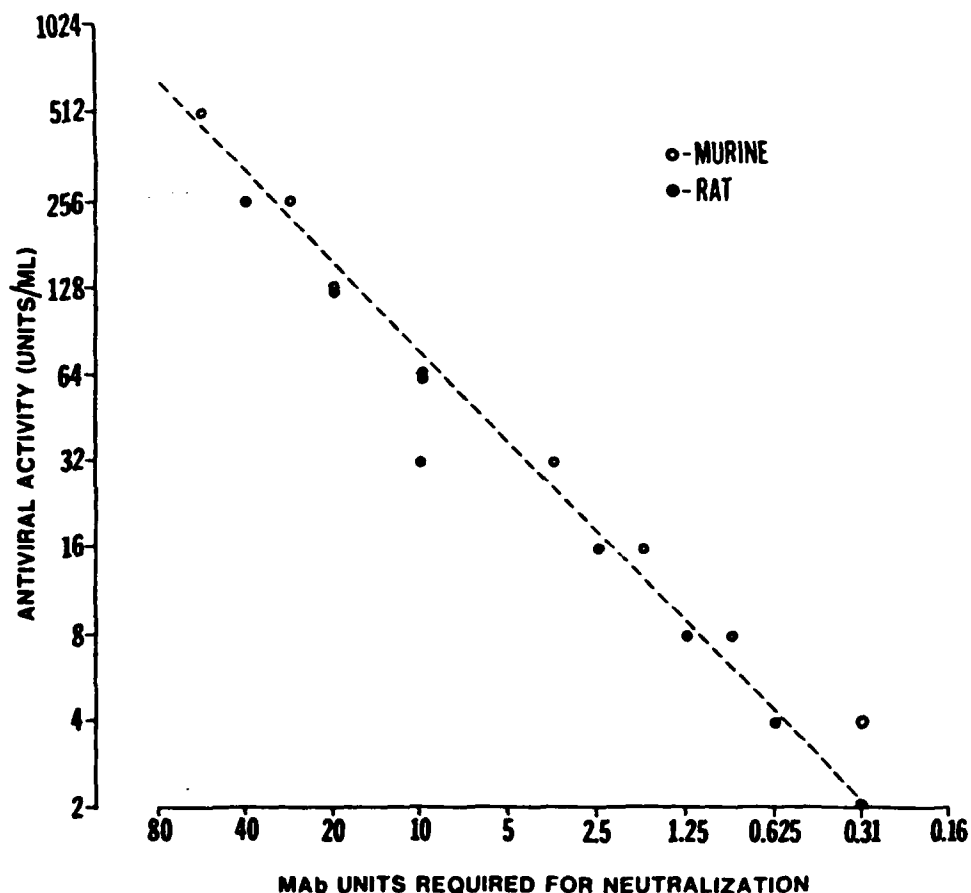
Spleen cells from DA rats injected 6 days earlier with  $5 \times 10^6$  Listeria monocytogenes subcutaneously, were suspended at  $10^7$  cells/ml in RPMI 1640 with 10  $\mu$ g/ml gentamycin (Schering) and stimulated with 5  $\mu$ g/ml PHA (Burroughs-Wellcome) for 24hrs.

Human IFN $\gamma$  induced by PHA induction of blood leucocytes and generously supplied by Flow Laboratories, Inc.



Fig. 2

Relationship Between Amount of MAb Required to Neutralize Different Concentrations of MuIFN $\gamma$  Antiviral Activity on Murine and Rat cells.



3. Production and Purification of Rat anti-MuIFN $\gamma$  MAb. The rat-anti MuIFN $\gamma$  MAb is currently being used to determine what effects MuIFN $\gamma$  has on macrophages and eventually will be used *in vivo* to establish what role(s), if any, this lymphokine may mediate in the generation and/or expression of cell-mediated immunity. To achieve these goals, large quantities of high-titered purified MAb will be required. Fortunately, the transspecies hybridoma (R4-6A2) has proven stable and is being propagated both in culture and as ascites in nude (nu/nu) mice. The

average neutralizing titer/ml of ascitic fluids is  $2 \times 10^5$  neutralizing units against 10 MuIFN $\gamma$  antiviral units, which is 1000-times more potent than that obtained *in vitro* under optimal culture conditions. Through means of radial immunodiffusion, it has been established that approximately 25% of the total ascitic fluid protein (60mg/ml) content is rat IgG $_1$  protein.

A number of different methods have been used to attempt the purification of the rat IgG. These have included  $(\text{NH}_4)_2\text{SO}_4$  precipitation, Protein-A affinity chromatography, as well as antibody affinity chromatography. Anion exchange chromatography using DEAE-Biogel has proven very satisfactory due both to the tremendous binding capacity of the exchanger for protein (45mg/1ml beads) and the mild conditions used to elute the MAb from the column. Based on theoretical considerations, it is currently believed, that this procedure may result in the total purification of IgG. Current studies are underway to determine if the rat MAb has been purified to homogeneity, for if this has been achieved, it will allow us to use commercially available rat IgG protein of similar protein content to that of the MAb for a mock preparation in future *in vivo* studies.

4. The effect of MAb on the macrophage activation potential of lymphokine preparations. Evidence indicates that IFN $\gamma$  can activate macrophage tumoricidal activity. One unresolved question is whether this is the only lymphokine affecting macrophage tumor cytotoxicity. To establish if MuIFN $\gamma$  is responsible for this activity, several experimental procedures were performed to determine whether the rat-anti MuIFN $\gamma$  MAB could eliminate the ability of crude lymphokine preparations to activate macrophages to destroy tumor cells *in vitro*.

a. Neutralization Studies. To test whether the rat anti-MuIFN $\gamma$  MAB possessed the ability to neutralize the macrophage tumoricidal activity of a different lymphokine preparations, an *in vitro* macrophage tumoricidal assay based on the one originally described by Pace and Russell was developed. Macrophages were harvested from the peritoneal cavities of AB6F $_1$  mice injected 3 days earlier with 1.0 ml of a 10% solution of Proteose-peptone. These cells were washed and resuspended at  $10^6$  cells/ml in RPMI-1640 medium supplemented with sodium pyruvate and 5% FCS. To wells of a microtiter plate 0.2 ml of cells were allowed to adhere. Two hrs later, non-adherent cells were washed off and 0.2 ml of test lymphokine preparations were added to groups of triplicate wells. Prior to their addition to the macrophage cultures, the test lymphokine preparations were reacted with and without varying concentrations of the MAB for 1 hr and then incubated with the macrophages in the presence of 10ng of endotoxin. Five hrs later,  $^{51}\text{Cr}$  labeled tumor target cells (P815 mastocytoma) were added to each well at an effector to target ratio of 5:1. The uppermost 0.1ml of fluid from each well was collected 16 hrs later and the tumoricidal activity was measured as % specific  $^{51}\text{Cr}$  release. It should be mentioned that the quantity of MuIFN $\gamma$  used in these macrophage activation studies was the lowest amount of antiviral activity (units/ml) which consistently induced the maximum expression of tumor cytotoxicity.

The results of these studies are presented in Table 2, where it is evident that crude, partially purified (Con A-bound) and highly purified (recombinant :  $10^7\text{U/mg}$ ) MuIFN $\gamma$  preparations all elicited macrophage tumoricidal activity. In contrast, a MuIFN $\alpha/\beta$  preparation induced only marginal activity when incubated at a 10-fold higher concentration than that required for MuIFN $\gamma$  preparations to induce maximum cytolysis. When test lymphokines were preincubated for 1 hr with twice the amount of MAB required to neutralize 50% of the stated MuIFN $\gamma$  concentrations, the antiviral activity was abolished, while the macrophage tumoricidal activity was

TABLE 2

7.

Inhibition of Lymphokine-induced Macrophage-  
Tumoricidal Activity by MAb to MuIFN

| IFN Preparation   | No MAb   | Relative excess of MAb <sup>a</sup> |                          |          | IFN titer <sup>h</sup> | (Units/ml)<br>+excess MAb |
|---|----------|-------------------------------------|--------------------------|----------|------------------------|---------------------------|
|   |          | 2x                                  | 5x                       | 10x      |                        |                           |
| Con-A-induced MuIFN $\gamma$ <sup>b</sup>                         | 30.8±1.4 | % Specific<br>21.4±3.4              | 51Cr Release<br>14.2±0.5 | 10.5±1.2 | 8                      | <4                        |
| PHA-induced MuIFN $\gamma$ <sup>c</sup>                           | 31.3±1.4 | 23.0±1.4                            | 13.8±1.0                 | 11.1±2.3 | 8                      | <4                        |
| PHA-induced MuIFN $\gamma$ <sup>d</sup><br>(Con-A-Bound Fraction) | 29.1±3.3 | 17.5±2.9                            | 11.3±2.3                 | 10.5±1.7 | 16                     | <4                        |
| PHA-induced MuIFN $\gamma$ <sup>d</sup><br>(Con-A-Bound Fraction) | 33.4±2.8 | 26.7±1.2                            | 20.0±3.2                 | 13.6±3.1 | 8                      | <4                        |
| recombinant DNA MuIFN $\gamma$ <sup>e</sup>                       | 32.6±2.0 | 23.8±1.8                            | 14.7±2.1                 | 10.6±1.7 | 16                     | <4                        |
| MuIFN $\gamma$ +MuIFN $\gamma$ <sup>f</sup>                       | 6.3±1.2  | 4.0±2.0                             | -                        | -        | 128                    | 128                       |
| Con-A-induced MuIFN $\gamma$ <sup>i</sup>                         | 32.6±1.7 | -                                   | -                        | 35.3±2.7 | -                      | -                         |

<sup>a</sup> The quantity of MAb reacted was based on neutralization of the antiviral activity of each preparation. Since the antibody titer is determined by the inhibition of 50% of the activity (see legend of Table 1), it was necessary to add at least 2-fold more antibody to abolish all antiviral activity.

<sup>b,c,d,e</sup> Lymphokines prepared according to methods outlined in legend of Table 1.

<sup>f</sup> Pure IFN $\gamma$ +3 was produced by New Castle disease virus-induced mouse C-243 cells and was purchased from Enzo Biochem.

<sup>g</sup> % specific <sup>51</sup>Cr release =  $\frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$

Total cell-associated <sup>51</sup>Cr determined by lysing P815 target cells with Triton X-100. Mean of triplicate samples for three separate experiments ± standard error of the mean.

<sup>h</sup> The IFN titer is defined as the reciprocal of the highest dilution of the sample which protects 50% of the assay cells (L929 B) from viral (Vesicular stomatitis virus) cytopathic effect.

<sup>i</sup> Macrophages were incubated with lymphokines for 5 hours prior to the addition of MAb.

inhibited 25-40%. In order to reduce the tumoricidal-inducing activity of all lymphokines below 50% of maximum activity, 5-10 fold more of the MAb than that needed to neutralize antiviral activity was required. At a 100-fold excess of MAb, the tumoricidal activity was reduced by 90% (results not shown). Addition of MAb to the macrophages following a 5 hr incubation with lymphokines did not inhibit tumoricidal activity. Why more MAb is required to neutralize tumoricidal activity than antiviral activity is not known, but may be related to the greater sensitivity of the tumoricidal assay.

b. Immunoabsorption of the macrophage activating factor. Anti-MAb affinity chromatography was used to demonstrate that MuIFN $\gamma$  is the lymphokine which induces macrophages to become tumoricidal. The MAb was covalently coupled to Sepharose 4B beads which were then used as an immunoabsorbent to specifically bind and thus delete MuIFN $\gamma$  from different preparations (Table 3). This procedure removed all the antiviral and macrophage tumoricidal activities from the different lymphokine preparations. Mock Sepharose beads failed to remove these activities from the different preparations.

These studies demonstrate that a rat MAb against MuIFN $\gamma$ , initially screened for neutralization of antiviral activity, can also neutralize the ability of lymphokine preparations to activate macrophage tumoricidal activity *in vitro*. However, to achieve this effect, relatively more MAb was required than was needed to neutralize antiviral activity. Similar results were obtained whether unfractionated lymphokines, partially or highly purified MuIFN $\gamma$  preparations were used. Published studies have shown that a MAb against human IFN $\gamma$  was capable of completely inhibiting not only antiviral activity, but also the ability of these preparations from activating the secretory and microbicidal functions of peripheral blood monocytes. The results of the combined studies with MAb's to mouse and human IFN $\gamma$ , plus our findings that immobilized MAb specifically removed the factor capable of activating macrophage tumoricidal activity from lymphokine preparations, indicates that the induction of macrophage activation and expression of antiviral activity are mediated by the same molecule or by molecules sharing similar epitopes.

TABLE 3

Specific Binding of Lymphokine-induced Antiviral and  
Macrophage Tumoricidal Activities to Immobilized Anti-IFN $\gamma$  MAb

| MuIFN $\gamma$ Preparation                             | Activities of lymphokines before and after incubation with<br>MAb coupled to Sepharose beads <sup>a</sup> |       |   |                |
|--|---|-------|---|----------------|
|  | Antiviral <sup>g</sup><br>(IFN titer)   |       | Tumoricidal <sup>f</sup><br>(% specific <sup>51</sup> Cr Release) |                |
|  | before  | after | before  | after          |
| Con-A - induced <sup>b</sup>                           | 128   | <4    | 34.6 $\pm$ 1.8  | 1.3 $\pm$ 1.1  |
| PHA - induced <sup>c</sup>                             | 128   | <4    | 34.4 $\pm$ 2.0  | 1.5 $\pm$ 2.3  |
| PHA - induced <sup>d</sup><br>(Con-A-Bound Fraction)   | 128   | <4    | 33.8 $\pm$ 1.5  | 1.7 $\pm$ 1.3  |
| PHA - induced <sup>d</sup><br>(Con-A-Unbound Fraction) | 128   | <4    | 34.4 $\pm$ 2.0  | 1.5 $\pm$ 0.7  |
| recombinant DNA <sup>e</sup>                           | 128   | <4    | 35.0 $\pm$ 2.1  | 4.9 $\pm$ 3.1  |
|  | Mock Sepharose beads without coupled MAb <sup>a</sup>   |       |   |                |
| PHA - induced <sup>d</sup>                             | 128   | 128   | 33.2 $\pm$ 1.5  | 34.1 $\pm$ 2.2 |
| recombinant DNA <sup>e</sup>                           | 128   | 96    | 32.9 $\pm$ 4.4  | 31.6 $\pm$ 3.4 |

a Lymphokines were diluted to 128 IFN U/ml, mixed either with MAb coupled to Sepharose beads or mock Sepharose beads and rotated for 15 hrs. at room temp. The beads were then pelleted at 200xg for 5 min. and the supernatant tested for antiviral and tumoricidal-inducing activities. Mock beads were generated by treating CNBr-activated Sepharose-4B, in the absence of protein, under identical conditions used for the coupling of MAb.

b,c,d,e Lymphokines prepared according to methods outlined in legend of Table 1.

f See legend of Table 2 for description of tumoricidal assay.

g See legend of Table 2 for definition of IFN titer.

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