immunofluorescent technique detecting surface immunoglobulins.[13] Enumeration of T lymphocytes was determined using a rosetting technique with neuraminidase-treated sheep erythrocytes[14]

To determine the ability of B lymphocytes to produce immunoglobulins *in vitro* cells were cultured with pokeweed mitogen for seven days and cytoplasmic immunoglobulins were detected by an immunofluorescent method.[15] Cellular immunity was assessed by delayed hypersensitivity skin testing with purified protein derivative (PPD), coccidioidin, trichophyton, candida, tetanus and streptokinase-streptodornase antigens.

Evaluation of Suppressor Cell Activity

a. Peripheral mononuclear cells were obtained by ficol-hypaque gradient centrifugation from the patient's peripheral blood and from a normal PPD skin test positive control. Viability of the cells was checked with trypan blue with greater than 90% viable cells. Cells at concentration of 1×10^6/ml were cultured in quadruplicate using microtiter plates in RPMI-1640 medium with L-Glutamine, penicillin-streptomycin and human AB serum 5% added. The plates were incubated in 5% CO_2 at 37°C for six days. Eighteen hours before harvesting 1 microcurie H^3 thymidine was added to each well. Cells were harvested using an automatic harvester and counting was done with a scintillation counter. The count was expressed as ΔCPM = experimental count per minute minus control.

b. Cells were cultured in medium alone, with PPD, and with and without indomethacin. Co-cultures were done with an equal amount of cells of the patient and the positive PPD control, also cultured in medium alone, with PPD, and with and without indomethacin. A dose response curve was determined for the response to PPD. The optimal dose of PPD is 20 micrograms/ml.

Indomethacin was prepared fresh as follows: indomethacin 35.7 mg was mixed with sodium carbonate anhydrous 11.4 mg and H_2O 10 cc. The solution was diluted to 10 micrograms/ml concentration.

c. *Separation of Adherent and Non-Adherent Cells.* Cells were allowed to adhere on glass for one hour in 5% CO_2 incubator at 37°C. Non-adherent cells were recovered by aspiration. After washing with RPMI-1640 three times, cells which adhered to glass were dislodged with a rubber policeman and both adherent and non-adherent cells were reconstituted in medium to a final concentration of 1×10^6 cells/ml. The ratio of adherent to non-adherent cells recovered was 1/2.

d. *Mixed Lymphocyte Cultures (MLC).* Allogeneic response of the patient's lymphocytes was studied using mixed lymphocyte cultures. The patient's cells (5×10^6ml) as stimulating cells were treated with mitomycin (25 micrograms/ml) for 30 minutes at 37°C followed by washing in RPMI-1640 medium three times, then reconstituted at final concentration of 1×10^6 cells/ml. Normal positive PPD control cells were prepared the same way.

Mixed lymphocyte cultures were done with both untreated patient's cells and control cells (two-ways MLC) and mitomycin-treated patient's cells with control cells and vice-versa (one-way MLC).

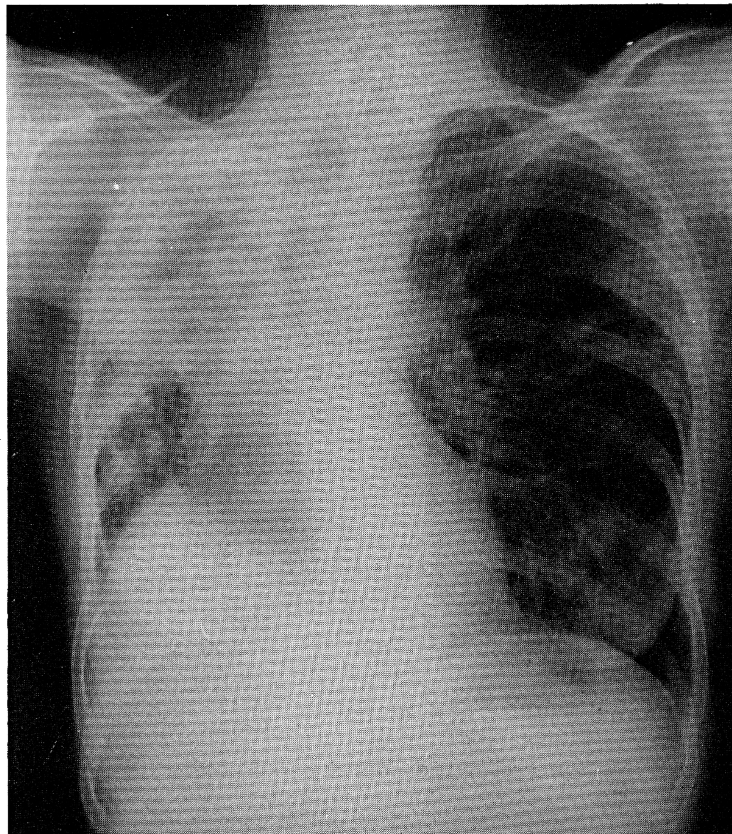

Figure 1. Chest radiograph showing active M. avium-intracellulare pulmonary disease.

Results

The serum immunoglobulins were all extremely low except for IgA (Table I). Enumeration of T and B cells showed a normal number of B cells (7.7% with control 9%), as usually is seen in common variable hypogammaglobulinemia. The T cells were minimally decreased (65% with control 83%). The ability of the B-lymphocytes to produce immunoglobulins *in vitro* upon stimulation with pokeweed mitogen was markedly impaired, as evidenced by a minimal number of positive cytoplasmic Ig cells: 0.5–1% as compared with the control 8–12%. Delayed hypersensitivity skin tests to all recall antigens including PPD were negative.

The patient's cells had no response to the PPD antigen in contrast to cells from the positive PPD control. The addition of indomethacin 10 micrograms/ml to the culture had no significant effect (Table II). In the mixed lymphocyte cultures, the mitomycin-treated patient's cells served as stimulating cells to the positive PPD control cells which proliferate normally to the allogeneic stimulus; however, the patient's cells had a poor allogeneic response to the mitomycin-treated positive PPD control cells (Figure 2). Therefore, most of the total allogeneic response in the two-ways mixed lymphocyte culture can be attributed to the proliferative response of