Leukocyte Migration Inhibition in Methotrexate-Induced Pneumonitis*  
Evidence for an Immunologic Cell-Mediated Mechanism  
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Methotrexate-induced pneumonitis is a well-known clinical entity, but the mechanism for the induction of the pulmonary disease is ill defined. In three patients with this disorder, evidence was obtained for elaboration of a lymphokine, leukocyte inhibitory factor (LIF), by peripheral blood lymphocytes after incubation with methotrexate (MTX) in the direct leukocyte migration inhibition test. Control lymphocytes from normal subjects, as well as from patients receiving methotrexate but without pneumonitis, failed to elaborate LIF in the presence of the drug in this test. Along with these results, we obtained bronchoalveolar lavage (BAL) cell data displaying high grade lymphocyte alveolitis with a lymphocyte subset inverted ratio. This production of LIF suggests that pneumonitis associated with methotrexate therapy is also associated with a specific cellular immune response to the drug.

Material and Methods

Patients

Three nonsmoking women 42, 18, and 62 years of age, with respectively, choriocarcinoma, acute myelogenous leukemia, and breast cancer, where given methotrexate weekly (mean dose: 24 mg; range: 15-30) over a period averaging 42 days (range: 35-49). The exact weekly dosing schedules and duration of treatment were respectively 30 mg and five weeks for patient 1, 15 mg and six weeks for patient 2, and 25 mg and seven weeks for patient 3. Injections were made, respectively, intramuscularly, intrathecally, and intravenously. A few days after the last injection, they complained of fever and dyspnea at effort. Chest x-ray films showed extensive alveolar and/or interstitial opacities in both lungs; hypoxia was present (mean level: 50 mm Hg, ie, 6.65 kPa). Bronchoalveolar lavage showed lymphocytic alveolitis (mean lymphocyte percentage: 61 percent) with, in two cases, an OKT4:OKT8 lymphocyte ratio of 0.50 and 0.43 (normal: 1.8 ± 0.7). Recovery occurred (on the 14th, 10th, and 18th days, respectively) (mean delay: 14 days) when methotrexate treatment was stopped.

Leukocyte Migration Inhibition Test in Presence of Methotrexate

Heparinized (20 U/ml) peripheral blood was used for the leukocyte migration inhibition (LMI) test. This test was performed respectively on the 8th, 15th, and 8th days after the onset of the pneumonitis. Leukocytes of each patient were isolated by centrifugation on Percoll gradients. After washing and counting, granulocytes of each subject were mixed with her own lymphocytes in a ratio of 2:1 respectively, in case 2, one half of the granulocytes were mixed with lymphocytes from a healthy control subject, and the other half with the patient's own lymphocytes. Agarose microdroplets containing mixed cell suspension were placed in wells of sterile disposable polystyrene plates. Cells were cultured in RDM 1640 medium containing 20 mM HEPES buffer, 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 percent fetal calf serum, hereafter called cell culture medium.

We used cell culture medium without methotrexate and cell culture medium with different concentrations of the drug (10 logs; from 10^-2 through 10^6 µg/ml): these in vitro methotrexate concentrations are close to therapeutic plasma levels of methotrexate usually registered. Leukocyte migration from agarose microdroplets was quantitated for each methotrexate concentration with a photoelectric procedure previously described. No other causes of pulmonary disease of acute onset were found: clinical and laboratory investigations for a bacterial, viral, fungal, parasitic, or malignant process were negative. Cell migration in medium with methotrexate was compared with migration of the same cells in medium without the drug; inhibition of leukocyte migration (LMI) was calculated in percentage by comparison with migration in medium without the drug; a curve was then obtained by plotting percent LMI against methotrexate concentration (log). Migration evaluation and calculations were done on 12, 24, 36, and 72 hour cultures; maximal inhibitions were observed by the 24th or the 36th hour. A LMI of more than 30 percent was considered significant. Variation in migration of control microdroplets was less than 10 percent; a variation of 2 percent of droplet size was due to the device used. This

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Manuscript received March 18; revision accepted July 8.  
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Leukocyte Migration Inhibition (Akoun et al)
test was performed in our three patients. It was simultaneously performed in six normal subjects ("healthy controls") and in three other patients with breast cancer and on methotrexate treatment but who were free from pneumonitis ("controls + methotrexate").

RESULTS

The most demonstrative LMI in patients with pneumonitis was observed in presence of concentrations of methotrexate as low as $5 \times 10^{-8}$ to $5 \times 10^{-7}$ M, that is, $10^{-3}$ µg to $10^{-2}$ µg/ml. No significant inhibition in presence of such low concentrations of drug was observed in healthy control subjects or in patients receiving methotrexate treatment but free from pneumonitis. Table 1 displays the most demonstrative LMI values. Statistically significant inhibition in patients with pneumonitis ($p<0.02$ to 0.05) is present, as compared to the control groups, at $10^{-4}$ and $10^{-3}$ µg per ml (ie, $5 \times 10^{-8}$ M and $5 \times 10^{-7}$ M, respectively).

Migration inhibition curves of three individual patients are shown in Figure 1. The three migration inhibition curves corresponding to the three groups of subjects are shown in Figure 2. The mean percentage of inhibition ($\pm$ SEM) is noted on the vertical axis for each concentration of methotrexate, indicated on the horizontal axis (log). The LMI in the three patients with pneumonitis is seen to occur as a bell-shaped curve, visible at log $-5$, $-4$, and $-3$ (ie, $5 \times 10^{-6}$, $5 \times 10^{-5}$, and $5 \times 10^{-4}$ M, respectively), absent in the two control groups. A second zone of inhibition distant from the first by three logs appears at 10 µg/ml (log 1 ie, at $5 \times 10^{-3}$ M) in both patients and healthy control subjects. Inhibition in this zone increases with drug concentration and is presumably related to cellular toxicity. In patients receiving methotrexate treatment but free from pneumonitis, this toxicity causes some stimulation of migration prior to inhibition (not shown in Figure 2).

The LMI in presence of methotrexate was observed with mixed cell suspension, containing both lymphocytes and granulocytes. In case 2, when one half of the granulocytes were mixed with lymphocytes from a healthy nonsensitized control subject, inhibition of migration was no longer observed (Fig 1), suggesting the requirement of factor(s) from immune lymphocytes for migration inhibition.

DISCUSSION

Methotrexate-induced pneumonitis has long been known. Its diagnosis is sometimes difficult, and this could have justified the need for lung biopsy; however, the pathologic findings are rarely specific and the procedure potentially harmful. Bronchoalveolar lavage cell data are of diagnostic interest when exhibiting lymphocytic alveolitis and lymphocyte subset imbalance. The results of the leukocyte migration inhibition (LMI) test reported herein would seem to help establish diagnosis and understanding of the disease.

Some recent reports have drawn attention to the positive results of this investigation in different cases of drug-related pneumonitis due to amiodarone, nitro-
Methotrexate seemed to have selectively stimulated leukocyte inhibitory factor (LIF) production in our cases. It should be noted that antigen-induced production of this lymphokine by lymphocytes is considered to be carrier-specific and not facilitated by hapten alone. However, it should be noted here that LIF production has also been observed in berylliosis, a well-documented metal-hypersensitivity disease. Similarly, the LMI test demonstrated LIF secretion induced by α-gliadin in patients with celiac disease. These inducing factors seem to be able to bind with serum proteins to make complete antigens. Because serum is present in the culture medium, this should have some facilitative effect in the stimulation of lymphocytes by incomplete antigens.

The specificity of our results is suggested by the lack of LIF production by lymphocytes from six normal subjects and three patients receiving methotrexate treatment but without interstitial pneumonitis. In addition, it should be underscored that in the LMI test for patient 2 in which patient lymphocytes were replaced by control lymphocytes from healthy nonsensitized control, no inhibition was observed (Fig 1).

Finally, the clinical impression that methotrexate-related interstitial pneumonitis is due to an immunologic hypersensitivity mechanism appears to be confirmed by our data. Since lymphokine release is primarily a T-cell function, cell-mediated immune phenomenon may play a major role in the pathogenesis of this disease.

ACKNOWLEDGMENT: We are grateful to Ms M. Michalon for her secretarial assistance and to Ms A. Bellaiche for blood samples from control group patients.

REFERENCES

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![Figure 2. Logarithmic plot of leukocyte migration inhibition in three groups of subjects. Concentrations of methotrexate are indicated on the horizontal axis and percentage of migration inhibition on the vertical axis.](image-url)
23rd Annual Arizona Chest Symposium

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