A nearly complete inhibition of protease surface activity was achieved by addition of purified α1-AT at a concentration of 0.2 mg/ml (table 2). At the same α1-AT concentration, the total proteolytic activity was only partially suppressed. The inhibition of lymphocyte surface proteolytic activity by α1-AT is in agreement with the demonstration of a reversible binding of α1-AT to tonsil cells incubated in serum-free medium[14], and with the reported binding of other protease inhibitors, like trasylol[3] or soybean trypsin inhibitor[14], to the lymphocyte surface. Since several synthetic or natural protease inhibitors are known to prevent lymphocyte blastogenesis, the demonstration of the suppression of lymphocyte surface proteolytic activity by α1-AT provides additional indirect evidence for the role of protease-anti-protease systems in the control of lymphocyte activation.

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Lymphocyte reactivity in Imuran-treated guinea-pigs and in vitro effect of colchicine

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Summary. Imuran treatment significantly diminished macrophage migration inhibition in immunized guinea-pigs and the percentage of E-rosette forming cells in all treated animals. 10^{-6} M colchicine in vitro significantly increased the percentage of E-rosette forming cells in Imuran-treated animals. The results suggest possible alteration of the lymphocyte receptors after treatment with Imuran.

The effect of Imuran (azathioprine) on some immune reactions is well established. However, the mechanisms of the action of this drug on lymphocyte reactivity are still not well understood. Winkelstein's[2] data suggest that the suppressive effects of this immunosuppressive drug are connected with its cytostatic effects. However, Bach[3] states that such effects of Imuran are not its only mode of action. He suggests that Imuran modifies the surface receptors on lymphocytes and thus alters their function.

The role of microtubules in the receptor distribution has been suggested by many authors in recent years[4-7]. Using antimicrotubular agents, Wybran[6] has shown that they affect human lymphocyte reactions with sheep erythrocytes in forming E-rosettes.

The effect of Imuran on lymphocyte reactivity using the macrophage migration inhibition test and the rosette forming test in ovalbumin-immunized guinea-pigs was investigated in this paper. The in vitro effect of colchicine, an antimicrotubular agent, on the formation of rosettes was studied as well.

Material and methods. Guinea-pigs of both sexes, 250–300 g b.wt, were used in the experiments. The animals were divided into 6 groups: the 1st group (I) consisted of 19 animals, which served as the control group and received 0.5 ml saline i.m. Group II (23 animals) received 0.5 ml of complete Freund's adjuvant (CFA) with 0.5 ml saline into the foot pads. Group III (23 animals) was injected with 5 mg of crystallized ovalbumin in 0.5 ml saline and 0.5 ml CFA into the foot pads. Group IV (10 animals) received 20 mg/kg b.wt of Imuran (Wellcome) in 0.5 ml saline i.m. Group V (10 animals) was injected with 0.5 ml CFA and 0.5 ml saline into the food pads and 20 mg/kg b.wt of Imuran i.m. simultaneously. Group VI (19 animals) received 5 mg of crystallized ovalbumin in 0.5 ml saline and 0.5 ml CFA, and 20 mg/kg b.wt of Imuran simultaneously.

Imuran was injected into the animals of the groups IV, V and VI on the day of the immunization and during 4 consecutive days.

The macrophage migration inhibition test (MMI) was performed according to David and David[8]. Briefly, peritoneal macrophages were washed and packed into capillary tubes which were cut at the cell-fluid interface. The capillaries were put in culture chambers containing tissue culture medium RPMI 1640 (Eurobio Paris), supplemented with antibiotics and 15% inactivated horse serum. Ovalbumin was added to the test chambers at a concentration of 80 μg/ml, while control chambers were without antigen. The chambers were incubated for 48 h at 37 °C and the migration index was calculated as:

$$\text{MI} = \frac{\text{migration area in test chambers}}{\text{migration area in control chambers}} \times 100$$

The E-rosette forming test (ERF) was performed according to Wilson[9] with some modifications: 0.25 ml guinea-pig lymph node lymphocytes (3×10^6 cells/ml) were mixed with 0.25 ml of 0.8% papain-pretreated rabbit erythro-

cytes[10,11] and 0.5 ml medium Parker 199 (Torlak, Beograd) supplemented with 5% fetal calf serum[12]. After incubation for 20 min at 37°C, the suspension was centrifuged and incubated at 4°C for 40 min. Rosettes with at least 3 erythrocytes were counted among 200 cells. To test the effect of colchicine on rosette formation, colchicine (Sigma) was added to the medium at a concentration of 10^{-6} M, and the E-rosette forming test was performed as in controls.

The tests were performed 5 days after immunization. Statistical analyses were performed using Student's t-test.

Results. The effect of Imuran on MMI and ERF is presented in figure 1. The animals of group III, which had been immunized with ovalbumin, developed a cell-mediated immune reaction to ovalbumin, as shown by the inhibition of macrophage migration. Nonimmunized animals of groups I and II showed no inhibition of macrophage migration and are presented as the controls in figure 1,a. The animals which had been immunized and simultaneously treated with Imuran showed no inhibition of macrophage migration, and the indexes of migration in this group were similar to the controls. The mean percentage value of ERF cells in normal guinea-pigs (group I) was 40%, and similar values were obtained in CFA-treated and in immunized animals (groups II and III). In all animals treated with Imuran, the percentage of ERF cells was significantly dimineshed (groups IV, V and VI) (figure 1,b).

When colchicine was added in the ERF test the percentage of ERF cells was not changed in the group of normal animals (group I). In animals treated with Imuran only, the presence of colchicine in vitro caused a significant increase in the percentage of E-rosettes (figure 2).

Discussion. The results obtained in our study show that ovalbumin-immunized animals develop a cellular immune reaction, as was evident in the MMI test. Simultaneous treatment with Imuran significantly diminished this reaction. This is in accordance with the results of other authors[14,15], who reported decreased reactivity in MMI skin reactivity, blast transformation etc, after Imuran-treatment, and with our earlier results[13]. Beside its effects on lymphocyte reactivity there is evidence[3,16] that Imuran diminishes the number of ERF cells. In our study we also found a significant decrease in the percentage of ERF cells in all animals treated with Imuran. However, the increase in percentage of ERF cells after in vitro treatment with colchicine suggests that the receptors for rabbit erythrocytes are present, but probably hidden on the lymphocytes from Imuran-treated guinea-pigs. Colchicine, as well as other antimicrotubular agents, affects the distribution of membrane receptors in various cells[4-7]. Our results support the opinion of Bach[3] that Imuran suppresses lymphocyte function not only by cytotoxic effects but also by alteration of the membrane receptors on the lymphocytes.

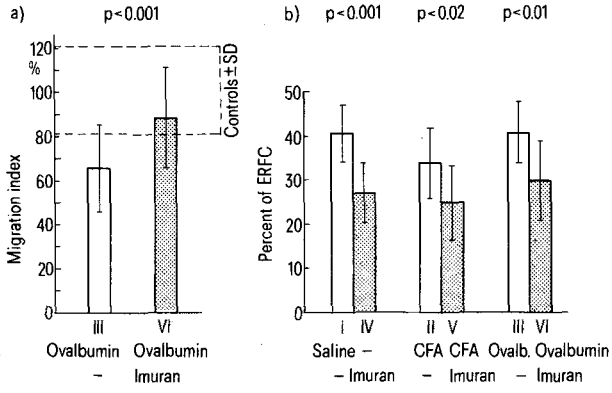

Fig. 1. The effect of Imuran on MMI and ERF *a* Mean values ± SD of the migration indexes of the immunized animals (group III ☐) and immunized and simultaneously Imuran-treated animals (group VI ▨). The area between the dotted lines represents the values obtained with the control animals (mean value ± SD). *b* Mean values ± SD of the percent of ERF cells (ERFC) in groups treated with Imuran (▨) and groups which were not treated with Imuran (☐).

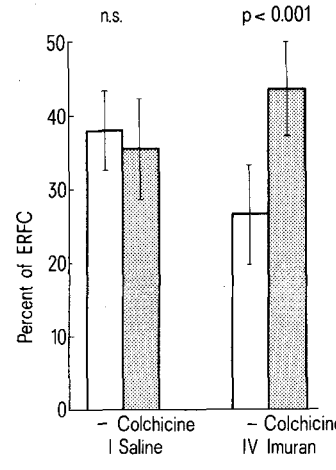

Fig. 2. Mean values ± SD of the percent of ERFC of the groups I and IV in presence of 10^{-6} M colchicine (▨) and without colchicine (☐).

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