later, a challenging dose of 2.5 mg of BSA in saline (containing 30×10^6 cpm of ^{125}I-BSA, New England Nuclear) was injected in the left knee. The right knee received an equal volume (0.1 ml) of saline and served as the internal control. One non-immunized rabbit was similarly challenged intra-articularly. This protocol was exactly repeated with another series of 10 animals.

Systemic immunity was evaluated at the time of arthritis induction by skin testing with 100 µg of BSA-saline in 0.1 ml[9] and by measuring hemagglutinating antibody to BSA-coated sheep erythrocytes[10]. These are expressed as $\log_2$ of the reciprocal titer. 4 weeks after the intra-articular challenge, animals were killed by exsanguination and both knees dissected. The synovial membrane histology was scored semi-quantitatively using preset criteria[5]. The results are expressed as a left to right score ratio. Cartilaginous structures (menisci and articular surfaces) were removed, weighed and assayed for retained radioactivity. The results are expressed as the left to right ratio. This ratio for a non-immune animal was 2.4. Actual radio-activity retained varied from 1×10^3 to 3×10^5 cpm.

Statistical evaluation of the data was made following principles established for small series[11].

Results and discussion. The data are shown in the Table. The host immune status is highly different in terms of cellular immunity ($p < 0.001$) and slightly different in terms of humoral immunity ($p < 0.01$). The IFA group did not get chronic synovitis while the CFA group did ($p < 0.001$). This confirms that humoral immunity is insufficient to generate a chronic inflammatory state. This experiment was designed to answer the following question: can local antigen retention be present without chronic inflammation? Although there is a difference in retention between the IFA and CFA groups, there is still a significant amount of retained radioactivity in animals that did not develop chronic synovitis. Furthermore, considering individual animals (not shown), there is a retention overlap between animals of both groups with the same amount of antibody, irrespective of the skin test and synovitis score. This would suggest that local retention is related to circulating antibody levels and not to synovitis. This conclusion was also reached in acute experiments[12].

In this model, we used a protein antigen, where it is expected that optimal humoral response will follow optimal cellular response. If chronic inflammation is mostly related to cellular immunity, and retention mostly related to humoral immunity, does it follow that synovitis is causally related to retention? Although all the measured parameters are significantly correlated, the two highest correlation are given by skin test and synovitis score on the one hand and antibody levels and retention ratio on the other hand. The worse correlation is between skin test or synovitis scores and retention values.

The present work shows that what is taken as intra-articularly trapped antigenic material (immune complexes) is not necessarily a chronic phlogitic stimulus in this animal model of arthritis.

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Effects of Antithymic Reticuloepithelial Cells Serum on the Levels of Circulating Thymic Factor and on the Sensitivity to Azathioprine of Spleen Spontaneous Rosette-Forming Cells

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Summary. Swiss mice treated with an antithymic reticuloepithelial cells serum (ATRES) showed a drastic and prolonged depression of the serum thymic factor. A similar but less pronounced effect was also observed following the administration of the antithymocyte (ATS) and the antilymphocyte (ALS) sera. Conversely, the azathioprine sensitivity of spleen spontaneous rosette-forming cells was highly modified by the ATRES but not by the ATS or the ALS. The probable mechanisms of such effects are discussed.

Ultrastructural and autoradiographic studies[3] have demonstrated the secretory activity of thymic reticuloepithelial cells. These cells have long been suspected of being the source of biologically active thymic factors, and some recent experiments support this hypothesis[4,5]. Bach et al.[6–9] have shown the presence in normal human and mouse sera of a factor produced by the thymus with thymosin like activity.

In the present work we approached this problem by using a specific antiserum prepared against thymic reticuloepithelial cells (ATRES). The effects induced by ATRES in Swiss albino mice, on the levels of serum thymic factor (TF), on the number of spleen spontaneous rosette-forming cells (sRFC), and on azathioprine (AZ) sensitivity of these cells, were studied, in order firmly to establish the epithelial thymic source of TF and some functional effects of this antiserum on the T lymphocytes spleen population. These effects were also compared to those provoked by an antithymocyte serum (ATS), an

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antilymphocyte serum (ALS) and a control normal rabbit serum (NRS).

Materials and methods. 4-week-old male Swiss albino mice were used. ATRES was prepared according to POTWOROWSKI's method[10]. The antiserum obtained was absorbed with mice lung homogenate, red blood cells and thymocytes. Its specificity against thymic reticuloepithelial cells was established by immunofluorescence.

For ATS and ALS preparation, thymocytes and lymphocytes, collected from the thymus and the lymph nodes respectively, were injected into New Zealand rabbits. The immunization schedule followed was essentially that proposed by LEVEY and MEDAWAR[11]; all sera were pooled, decomplemented and absorbed with red blood cells. The experimental procedure is reported in the legends to figures. Sera of each group of mice treated with different antisera and with NRS were pooled, filtered on Amicon filter UM 10 and tested for circulating TF according to BACH's method[12]. The number of spleen sRFC and their AZ sensitivity were evaluated according to the method reported by BACH et al.[13].

Results. The variations of serum TF among different experimental groups of animals are shown in Figure 1. On the 1st day after treatment with ATRES, a rapid fall of serum TF from 1/128 (normal value) to 1/8 was observed. This value remained constantly low for 14 days. Afterwards, serum TF increased again, reaching its normal value by the end of the experimental period (56 days).

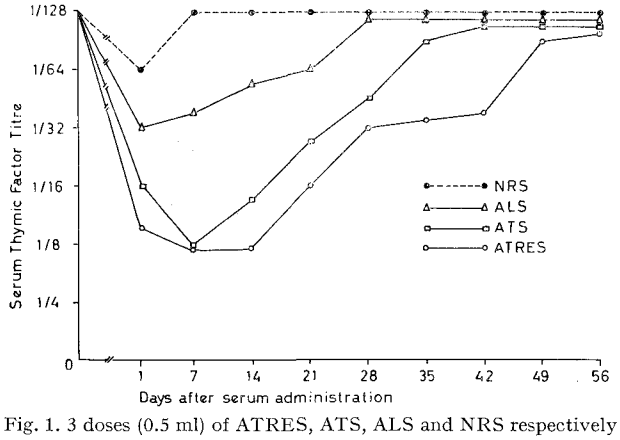

Fig. 1. 3 doses (0.5 ml) of ATRES, ATS, ALS and NRS respectively were administered in 3 consecutive days to 4 groups of mice, each composed of at least 8 animals. Sera of each group of mice were pooled and tested for the presence of the circulating thymic factor, beginning from the 1st day after the 3rd injection over 56 days.

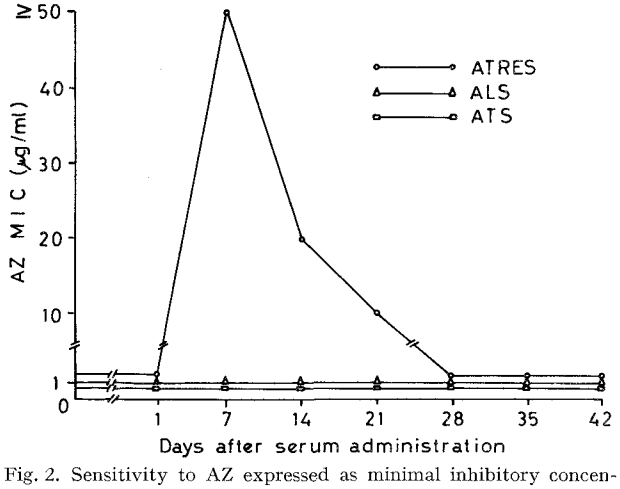

Fig. 2. Sensitivity to AZ expressed as minimal inhibitory concentration in µg/ml (MIC) of the spleen sRFC from groups of mice treated with ATRES, ATS and ALS as described in Figure 1.

In the group treated with ATS, the decrease of TF was of similar intensity but its restoration to normal value occured within 35 days. 1 day after ALS treatment, serum TF levels dropped to a titre of 1/32, which returned to normal within 28 days. Conversely, treatment with NRS only slightly modified serum TF levels, which were restored within 7 days.

Injections of either ATS or ALS induced also a significant drop in the number of spleen sRFC[14] which lasted 4 weeks after the treatment. In contrast no effects were noted on AZ sensitivity of spleen sRFC (Figure 2). ATRES injection did not modify the absolute number of spleen sRFC in the first 21 days (while afterwards a visible depletion of sRFC was noted), but the AZ sensitivity of sRFC was drastically decreased within 7 days and returned to standard values within 28 days (Figure 2). In the control group (NRS treated mice) spleen sRFC number and sRFC AZ sensitivity were not modified at all.

Discussion. In view of the specificity of ATRES, as demonstrated by the immunofluorescence test, our results clearly show the epithelial thymic origin of serum TF: in fact, ATRES is able to produce a drastic and prolonged decrease of this factor. Two mechanisms, which are not necessarily mutually exclusive, probably operate in inducing serum TF fall: 1. ATRES owing to its content of antibodies against TF directly inhibits circulating TF; 2. Thymic reticuloepithelial cells are inhibited in loco by ATRES[15], even though intrathymic penetration of ATRES may be questioned[16]. At present, it is not possible to know if the same mechanisms induce the fall of serum TF after ATS or ALS treatments because of the fact that reticuloepithelial cells can share common antigens with thymocytes[10] and, possibly, with lymphocytes T present in the lymph nodes. Additional (or alternative) mechanisms cannot be excluded, in view of the drastic lymphopenia induced by ATS and ALS; in any case, these mechanisms are less efficient than ATRES in inducing the TF decrease. The transient and slight decrease of the serum TF after NRS treatment might be caused by corticosteroids, which have been shown to inhibit serum TF production[6].

The variation of sensitivity to AZ of spleen sRFC makes it possible to demonstrate the selectivity of action of ATRES as compared with other antisera. The decreased sensitivity to AZ induced by ATRES must be considered in the light of the high dependence on thymic hormones of this spleen sRFC population[17], and is comparable to the effects of thymectomy[7]. At present it is not possible to decide whether this decreased AZ sensitivity, induced by ATRES, but not by ATS or ALS, is due to a modification of the characteristics of the T-RFC membrane, or to a drastic direct action on the thymus which could result either in an inhibition of the differentation process of thymocytes or in the production of thymic hormones.

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