Even lower concentrations of 5BdU appeared to cause a slight increase in numbers of erythroid cells formed in some experiments (e.g. Table, experiments 1 and 4). Further examination of this possibility established that maximum numbers of erythroid cells were consistently increased by about 40% in cultures of either cell population containing 0.2 μg/ml of 5BdU. Data for a representative experiment with cells of population EP2 are given in the Figure. Only slight and inconsistent stimulation was obtained at concentrations of 5BdU of 0.1 and 0.5 μg/ml, or in cultures of mechanically dispersed blastodiscs[9] at 0.2 μg/ml.

5BdU has been previously reported to suppress erythropoiesis by cells of the primitive streak chick blastodisc, both in cultures of mechanically dispersed cell 'miniclusters'[10] and in the intact blastodisc[5–7]. It has also been reported to suppress differentiation and hormone-elicited synthesis of specific proteins in a variety of experimental systems[11–21]. Conversely, 5BdU has been reported to induce differentiation of cultured neuroblastoma cells[22, 23].

However, we believe this to be the first recorded case of both inhibition and stimulation of differentiation in a single experimental system at different concentrations of 5BdU. It remains to be determined whether both effects upon erythropoiesis are due to effects of the nucleoside upon the same or different types of cell present in the heterogeneous cell populations.

[10] H. K. HAGOPIAN, J. A. LIPPKE and V. M. INGRAM, J. Cell Biol. *54*, 98 (1972).
[11] J. ABOTT and H. HOLTZER, Proc. natn. Acad. Sci., USA *59*, 1144 (1968).
[12] J. ABBOTT, R. MAYNE and H. HOLTZER, Devl. Biol. *28*, 430 (1972).
[13] J. R. COLEMAN, A. W. COLEMAN and E. HARLINE, Devl. Biol. *19*, 527 (1969).
[14] R. LASHER and R. CAHN, Devl. Biol. *19*, 415 (1969).
[15] S. SILAGI and S. A. BRUCE, Proc. natn. Acad. Sci., USA *66*, 72 (1970).
[16] R. H. STELLWAGEN and G. M. TOMKINS, J. molec. Biol. *56*, 167 (1971).
[17] F. STOCKDALE, K. OKAZAKI, M. NAMEROFF and H. HOLTZER, Science *146*, 533 (1964).
[18] R. W. TURKINGTON, G. C. MAJUMDER and M. RUDDLE, J. biol. Chem. 246, 1814 (1971).
[19] L. YOUNKIN and D. SILBERBERG, Expl Cell Res. *76*, 455 (1973).
[20] B. L. JAILKHANI and G. P. TALWAR, Nature New Biol. *239*, 240 (1972).
[21] B. T. WALTHER, R. L. PICTET, J. D. DAVID and W. J. RUTTER, J. biol Chem. *249*, 1953 (1974).
[22] D. SCHUBERT and F. JACOB, Proc. natn. Acad. Sci., USA *67*, 247 (1970).
[23] R. SIMANTOV and L. SACHS, Eur. J. Biochem. *55*, 9 (1975).

Is Local Antigen Persistence Responsible for the Chronicity of the Experimental Immune Arthritis of the Rabbit?

H. A. MÉNARD[1] and J. DION[2]

Rheumatic Diseases Unit, Immuno-rheumatology Laboratory, Medical School Center, Sherbrooke (Quebec, Canada J1H 5N4), 3 May 1976.

Summary. Local antigen trapping in the cartilaginous intra-articular structures can be present without histological evidence of synovitis and is therefore, not necessarily causally related to chronic inflammation.

A chronic arthritis can be induced in immunized rabbits by the intra-articular injection of protein antigens[3, 4]. The systemic humoral response to the antigen seems to be insufficient for the establishment of the chronic inflammatory state unless systemic cell mediated immunity to the antigen is obtained[4, 5]. Two hypotheses have been proposed to explain the chronicity. COOKE et al.[6] have suggested that the antigen persists, locally trapped in the cartilaginous intra-articular structures as an immune complex. This would provide the chronic phlogistic stimulus. GLYNN[7] on the other hand, suggests a locally induced auto-immune process, self perpetuation being the result of an immune response to tissue breakdown products generated during the original antigen induced inflammation. In this communication, we have studied the relationships of host immune status to chronic synovitis and to what has been taken as a measure of antigen persistence[6, 8]. The results suggest that local retention is always a feature of chronic synovitis but that it can be present without histological evidence of chronic inflammation.

Materials and methods. Male New Zealand White rabbits weighing 2 to 3 kg, were used. 2 groups of 5 animals each were immunized by footpad injections of 5 mg of bovine serum albumin (BSA, Sigma) in complete (CFA) or incomplete (IFA) Freund's adjuvant (Difco). 4 weeks

Immune status and chronic synovitis

Immunization (number of animals)	Skin test Score	Antibody ($\log_2$ titer)	Synovitis Score[a]	Retention Ratio[a]
BSA-CFA (7)	2.6±0.4	14.71±1.12	6.6±1.7	88 ±11.6
BSA-IFA (10)	0.6±0.1	11.55±0.47	0.8±0.2	32.7± 7.0
p-value	< 0.001	< 0.01	< 0.001	< 0.001

Values represent mean ± SE *p*-values are for the Student *t*-test.
[a] Ratio of left (BSA injected) to right (saline injected) knees.

[1] Address for reprints requests: H. A. MÉNARD, Assistant professor of Medicine, University of Sherbrooke, Rheumatology Department, C. H. U., Sherbrooke, Quebeck, Canada J1H 5N4.

[2] Acknowledgment. This work was supported by the Medical Research Council of Canada (No. MA–5061) and the Canadian Arthritis and Rheumatism Society (No. 15–180–(73). The authors wish to thank Mr. E. PICARD for his help with the animals, and Mrs. T. CARRIER for typing the manuscript.

[3] F. KLINGE, J. Beitr. Path. *83*, 185 (1929).
[4] D. C. DUMONDE and L. E. GLYNN, Br. J. exp. Path. *43*, 373 (1962).
[5] H. A. MÉNARD and J. DION, J. Rheumath. *2*, 373 (1975).
[6] T. D. V. COOKE, E. HURD, M. ZIFF and H. E. JASIN, J. exp. Med. *135*, 323 (1972).
[7] L. E. GLYNN, Ann. rheum. Dis. *31*, 412 (1912).
[8] T. D. V. COOKE and H. E. JASIN, Arth. Rheum. *15*, 327 (1972).

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.

[9] S. Leskowitz and B. H. Waksman, J. Immun. *84*, 58 (1960).
[10] G. H. Sweet and F. L. Welborn, J. Immun. *106*, 1407 (1971).
[11] D. Schwartz, *Méthodes statistiques*, 3rd edn. (Flammarion, Paris 1969).
[12] H. E. Jasin, Clin. exp. Immun. *22*, 473 (1975).

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

E. Garaci[1], Vera del Gobbo[2] and Cristina Rinaldi-Garaci[2]

Institute of Microbiology, Catania University, I–95100 Catania (Italy); and Institute of General Biology dell'Università, Piazzale delle Scienze, I–00100 Roma (Italy), 20 April 1976.

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

[1] Institute of Microbiology, Catania University, I–95100 Catania, Italia.
[2] Institute of General Biology dell'Università, Piazzale delle Scienze, I–00100 Roma, Italia.

[3] S. L. Clark, in *Thymus: Experimental and Clinical Studies* (Eds. G. E. W. Wolstenholme and R. Porter; J. and A. Churchill Ltd., London 1966), p. 3.
[4] H. Wekerle, J. R. Cohen and M. Feldman, Eur. J. Immun. *3*, 745 (1973).
[5] B. Mandi and T. Glant, Nature New Biol. *246*, 25 (1973).
[6] J. F. Bach, M. Dardenne and M. A. Bach, Transplant. Proc. *5*, 99 (1973).
[7] J. F. Bach and M. Dardenne, Transplant. Proc. *4*, 345 (1972).
[8] M. Dardenne, M. Papiernik, J. F. Bach and O. Stutmann, Immunology *27*, 299 (1974).
[9] J. F. Bach, M. Dardenne, S. M. Pleau and M. A. Bach, in *Thymus Factors in Immunity* (Ed. H. Friedman, The New York Academy of Sciences, New York 1975), p. 186.