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The origin of the apparent synovial lining cell hyperplasia in rheumatoid arthritis: evidence for a deep stem cell

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Summary. The experimental model of inflammatory arthritis in the rabbit has been used to study the possible origin of the apparent synovial lining cell hyperplasia. A small amount of ^{3}H-thymidine was injected into the joints and the fate of the labelled cells investigated by autoradiography. At times up to 24 h after the injection, most of the labelled cells occurred in the sub-lining region; this proportionality was reversed when the joints were sampled at later times. These results indicate that at least much of the increase of synovial lining cells may be derived from cells from deeper in the synovial tissue which move to the synovial surface.

Keywords: experimental allergic arthritis, synovial lining cell hyperplasia, autoradiography

Although apparent hyperplasia of the synovial lining cells and considerable thickening of the synovium are common features of rheumatoid arthritis, the origin of the greatly increased number of synovial lining cells is still unknown. Little evidence of mitosis, or of DNA-synthesis was found in human rheumatoid synovial tissue in the studies of Mohr *et al.* (1975) and of Coulton *et al.* (1980). Clarris *et al.* (1977) noted a remarkable lack of proliferative activity in cells derived from rheumatoid synovium placed in proliferative culture. In our own studies on over 2 000 specimens of human rheumatoid synovial tissue, taken at synovectomy at various times during the day, a few mitotic figures were seen in only one specimen. It was possible, however, that proliferative activity of synovial lining cells might be restricted to those times of day at which synovectomy is not normally done. Consequently we have studied the development of the chronic experimental allergic arthritis in the rabbit (Dumonde & Glynn 1962; Consden *et al.* 1971) which, although it is generally restricted to the challenged knee, bears remarkable similarity to the human condition, both histologically (Glynn 1972; Henderson *et al.* 1982*a*) and metabolically (Henderson & Glynn 1981). In earlier studies it was shown that there was considerable DNA-synthesis and proliferation during the acute phase, namely during the first three weeks after challenge (Henderson *et al.* 1981) although similarly increased proliferative activity was found in rabbits immunized with Freund's incomplete adjuvant (Henderson *et al.* 1982*b*). This work demonstrated that the synovial lining cells were capable of proliferative activity. However, following the acute phase, the proliferative activity declined markedly during the development, over the next 3 weeks and thereafter, of the chronic arthritis (Henderson *et al.* 1982*a*). By week 8

after challenge, the degree of DNA-synthesis was the same as in the synovial lining cells of control joints. Moreover, it was shown that, over a full 24 h period, the degree of DNA-synthesis, or of uptake of tritiated thymidine, was fairly constant.

Seeing that these studies had shown only that the hyperplasia of the chronic phase was unlikely to be due predominantly to proliferation of the synovial lining cells themselves, we have investigated the possibility that, to some extent at least, it might arise from recruitment from cells in the underlying stroma. Consequently we used the same rabbit model and injected ^{3}H-thymidine into the knee-joint 6 to 8 weeks after challenge. The amount injected corresponded to that used by Loewi *et al.* (1971)(confirmed by Henderson *et al.* 1981) which was shown to be restricted to the joint: no discernible label was found in the cells of the adjacent bone-marrow or nearby lymph-nodes (Henderson *et al.* 1981).

Materials and methods

Experimental procedure. Rabbits of the Old English strain (Cheshire Rabbit Farms, Tarporley, Cheshire) and of the New Zealand White strain (Buckstead Rabbits, East Sussex) of 1.5 to 2.5 kg body weight were immunized with ovalbumin (Sigma, grade V, crystallized and lyophilized) at a concentration of 5 mg/ml in an emulsion containing equal volumes of Freund's complete adjuvant and sterile saline. Subcutaneous injections (0.5 ml) were made between the scapulae and repeated 2 weeks later. Four to six weeks afterwards the animals were checked for delayed hypersensitivity. Then one knee joint was challenged by injecting 0.5 ml of a sterile solution of saline containing 20 mg/ml of ovalbumin. After about 6 to 8 weeks following challenge, during the chronic inflammatory phase, the affected joint was injected with 0.5 ml of sterile saline containing 10 μCi of tritiated thymidine (Amersham TRA 120). They were killed by intravenous Sagatal (pentobarbitone sodium: May and Baker), at various times thereafter.

Preparation of autoradiographs. Small blocks (approximately 3–5 mm^3) of the synovial tissue and of the lower end of the femur, were chilled by precipitate immersion in *n*-hexane (BDH 'low in aromatic hydrocarbons' grade, boiling range 67–70°C) and sectioned at 10 μm in a Bright's cryostat (cabinet temperature −25°C to −30°C and with the knife cooled with solid CO_2. The sections were 'flash-dried' on to slides that had been 'subbed' with chrome–alum–gelatine (Chayen *et al.* 1973) and dried overnight. They were then fixed in acetic–ethanol (1 : 3 v/v) for 20 min. Autoradiographs were prepared by the dipping film procedure, with Ilford K5 nuclear research emulsion and stored in the dark, at 4°C, for 8 weeks before being developed. They were then stained with haematoxylin and eosin.

Method of counting. The hypothesis underlying this study was the possibility that the hyperplasia of the synovial lining cells might involve recruitment from cells deeper in the stroma. Consequently we were required to assess how many labelled synovial lining cells occurred at various times after labelling, and how many labelled cells were present in line with these synovial lining cells but deeper in the stroma. To this end we used an eye-piece grid which consisted of two parts: one, 40 μm square in the plane of the section, to encompass a depth of about three synovial lining cells, and the other, also of 40 μm breadth, but 200 μm long (Fig. 1). The measurements were made with a Zeiss microscope with a ×25, NA 0.6 objective and ×10 eyepiece. In this way we could count the number of histologically definable labelled synovial lining cells and the number of labelled cells directly below these. The grid was moved to the immediately adjacent field and so on, so that the measurements encompassed all the length of the lining in each section, and the corresponding columns of tissue of the stroma. Each length of 40 μm of

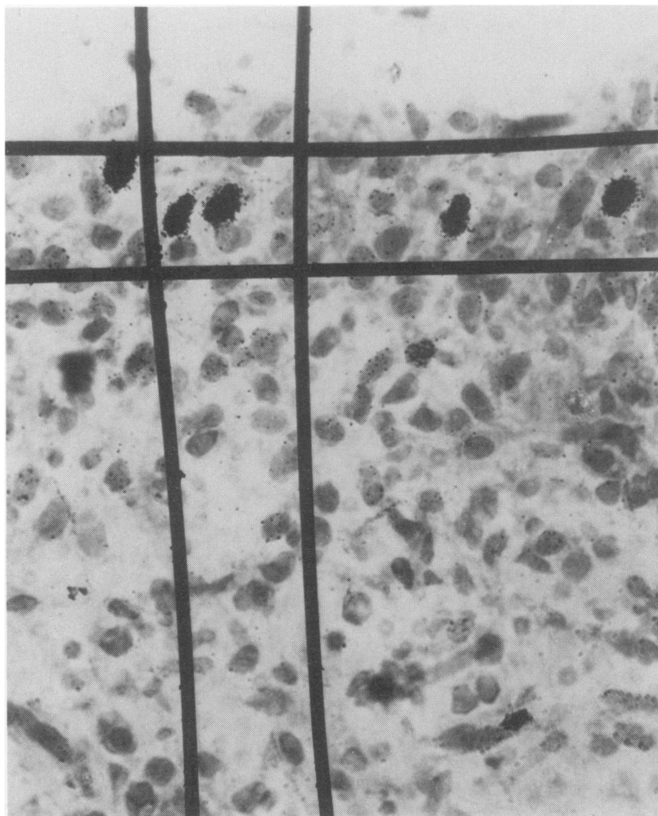

Fig. 1. An autoradiograph of synovium, stained with haematoxylin and eosin, and viewed with the eyepiece that contains the measuring graticule. The small square in the graticule is 40 × 40 μm, and contains two of the five labelled synovial lining cells seen in the surface layer in this section. The extension of this square is 40 μm by 200 μm for measuring the number of labelled cells in the synovium below the immediate lining layer. × 400.

histologically definable synovial lining cell surface was counted, whether or not it contained labelled cells. Thus if 40 fields were measured (as in Table 1), this corresponded to a length of 1600 μm of synovial lining, and its underlying stroma. The significance of the differences between the number of labelled cells was evaluated by the chi-square test.

Results

Validation of the intra-articular labelling

Autoradiographs prepared from cryostat sections of undecalcified lower end of femur from three injected joints contained no radioactive label, even in the bone marrow.

Distribution of label with time after injection

In general (Table 1) the counts of labelled cells made in serial sections, or in sections from different blocks of synovium from any one joint, gave similar results. In these counts, care was taken to exclude obvious chronic inflammatory cells (plasma cells and lymphocytes). In all sections taken up to, and including, 24 h after injection of the label, most of the labelled cells were found in the deeper stroma (Fig. 2; Table 1). It was

Table 1. Details of the number of labelled synovial lining cells and the number of labelled cells in the underlying stroma found at various times after injection of ^{3}H-thymidine into the joint

Rabbit	Time of labelling	Block or section	Number of fields measured	Number of labelled cells in:		Average number of labelled cells per grid in:		Number in deeper stroma: number in lining*
				lining*	deeper stroma	lining*	deeper stroma	
22	1 h	1	34	11	27	0.32	0.79	2.45
		2	40	20	39	0.5	0.97	1.95
		3	30	18	47	0.6	1.56	2.6
23	2 h	1	27	21	88	0.78	3.26	4.2
		2	38	27	112	0.71	2.96	4.1
27	2 h	1	8	2	7	0.25	0.88	3.5
21	24 h	1	11	14	28	1.27	2.54	2.0
		2	11	16	31	1.4	2.82	1.9
		3	11	17	27	1.5	2.45	1.6
72	3 days	1	36	11	10	0.3	0.27	0.9
		2	72	34	24	0.47	0.33	0.7
		3	46	32	23	0.7	0.5	0.72
73	3 days	1	77	44	10	0.57	0.13	0.22
		2	79	29	17	0.37	0.21	0.59
74	7 days	1	34	16	7	0.56	0.23	0.44
		2	21	3	4	0.14	0.19	1.3
		3	10	6	4	0.66	0.44	0.6
		4	36	14	7	0.39	0.19	0.5
		5	32	5	9	0.15	0.28	1.8

* Synovial lining cells.

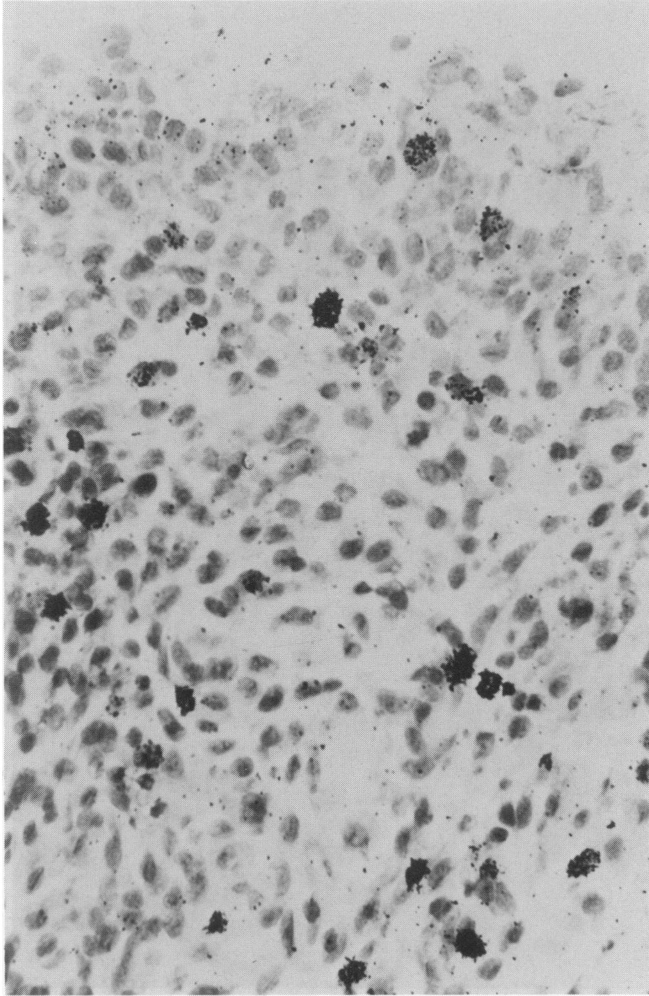

Fig. 2. An autoradiograph of a section from tissue taken 24 h after the injection of ^{3}H-thymidine. Most of the labelled cells lie in the deeper tissue below the synovial lining cell layer of three to five cells. Stained with haematoxylin and eosin. × 300.

difficult to define these stromal cells histologically, seeing that they were covered by a dense autoradiograph, but they appeared to resemble fibroblasts. In sections taken 3 days and 7 days after injection of the radioactive label, most of the labelled cells were synovial lining cells which were present in the superficial 40 μm of the sections covered by the smaller region of the grid (Fig. 3; Table 1). The difference between the number of labelled cells in the stroma as against the number of labelled synovial lining cells at the two main times of sampling was statistically significant ($P<0.001$).

Discussion

The normal synovium is lined by apparently differentiated cells which have been dis-

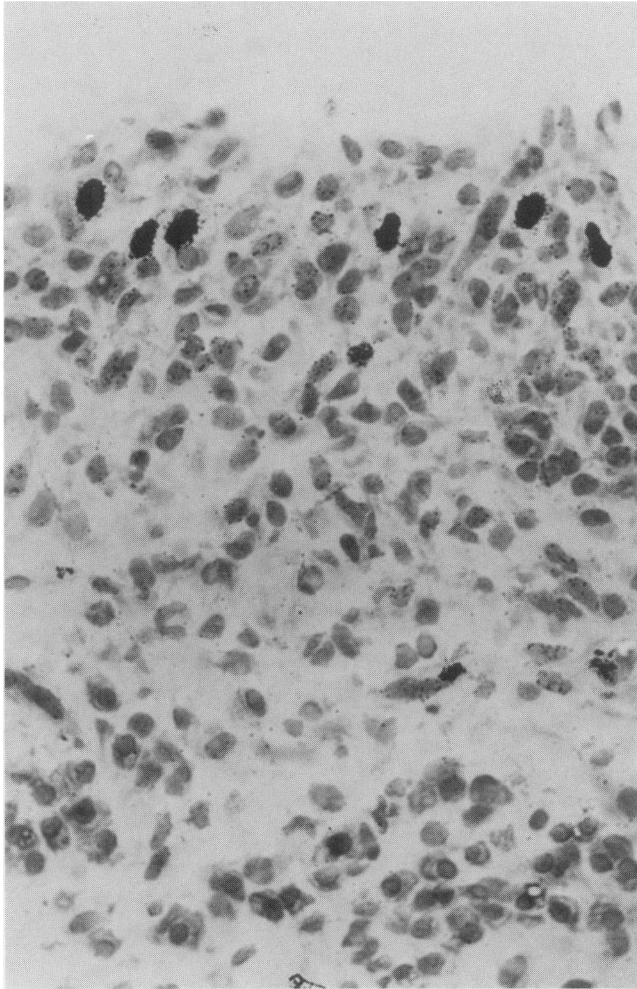

Fig. 3. An autoradiograph of a section from tissue taken one week after the injection of ^{3}H-thymidine: most of the labelled cells are synovial lining cells. Stained with haematoxylin and eosin. × 300.

tinguished into the macrophagic Type A and the fibroblast-like Type B cells (Barland *et al.* 1962). In life, these cells are shed into the synovial fluid from which they can be recovered in synovial fluid aspirates. The origin of these cells is still not clear. The origin of the vastly increased number of synovial lining cells in the rheumatoid joint is even less understood. However, as regards the normal joint, Edwards and Willoughby (1982) demonstrated that, in irradiated mice, cells derived from the bone marrow contributed to the A-cell population of the synovial lining. This work supported the earlier suggestion of Barratt *et al.* (1977). Edwards and Willoughby (1982) also suggested that the B-cells were not necessarily derived from the bone marrow and that these could have originated from division of some cell type lying deeper in the synovial stroma. Their experiments were not designed to test this hypothesis.

In contrast, our experiments were deliberately designed to test this possibility in

Table 2. Summary of the altered proportion of labelled synovial lining cells and labelled cells deeper in the stroma up to 24 h and at later times after the injection of ^{3}H-thymidine

Time of labelling	Average number of labelled cells per grid		Proportion of labelled cells
	lining*	deep	lining* : deep
Up to 24 h	0.81 ± 0.47	2.02 ± 0.98	1 : 2.7
3 & 7 days	0.43 ± 0.2	0.28 ± 0.12	1 : 0.78

* Synovial lining cells.

chronically inflamed synovium and to exclude the possible effects of irradiation. The amount of ^{3}H-thymidine that was injected intra-articularly had been shown by Loewi *et al.* (1971), by Henderson *et al.* (1981), and by our own study, to be sufficiently small that it did not label cells of the bone marrow even adjacent to the joint.

Our results showed that, in the specimens from the four rabbits sampled within 24 h of the injection of ^{3}H-thymidine, there were from about twice to four times the number of labelled cells in the column of stroma, measured within the long region of the grid (up to 200 μm from the surface 40 μm) as there were labelled synovial lining cells. In contrast, with the exception of one slide which had little label, in the specimens sampled at later times there was either a similar number of, or markedly fewer, labelled cells in the stroma than there were labelled synovial lining cells. Considering all the results together (Table 2), in sections of tissue sampled up to 24 h after injection of the label there were nearly three times as many labelled cells in the stroma as there were labelled synovial lining cells whereas this ratio changed to 0.78 to 1 in the later samples. In these there were fewer labelled cells, due to exfoliation from the surface, with accretion into the surface from cells from deeper in the stroma.

The results of the present studies indicate that, at least in this form of experimentally induced arthritis, the increased number of synovial lining cells can arise from recruitment from cells lying deeper in the stroma. It is just conceivable that the cells in the stroma that took up ^{3}H-thymidine might be bone-marrow derived macrophages that were undergoing cell-division in the synovium. We cannot say whether the labelled cells in this study were of Type A or B. Edwards and Willoughby (1982) suggested that only the Type B cells might arise from cells in the stroma. However, it has been suggested by Barratt *et al.* (1977) that the cells of the synovial lining belong to a single cell-type and that, under appropriate conditions, they can assume the A or B form. Thus the work of Edwards and Willoughby (1982) on the normal mouse joint and our results on experimentally induced arthritis in the rabbit indicate that the apparent hyperplasia of the synovial lining cells may arise both from bone marrow-derived macrophages and from division of a stem cell lying deeper in the synovium.

Acknowledgements

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