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Turnover of Tritiated-Thymidine-labeled Mononuclear Cells in Tuberculous Lesions of Rabbits: A Comparison of Primary Dermal BCG Lesions and Those of Reinfection[1] (39242)

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The use of a 24-hr dermal inflammatory reaction to trap mononuclear cells (MN) (macrophages and some lymphocytes)[4] from the blood stream has not been generally used to study MN turnover in chronic inflammatory lesions. Yet such a trap can chemotactically sample from the blood stream the very cells that participate in inflammation. This report utilizes this method to help determine MN turnover in primary dermal BCG lesions and those of reinfection (see Discussion).

Materials, methods and experimental procedures. An aqueous solution of tritiated thymidine (^{3}HT) (purchased from New England Nuclear Corp., Boston, Massachusetts) with a specific activity of 18 to 20 Ci/mmole was diluted with an equal part of 0.9% nonpyrogenic NaCl solution (Cutter Laboratories, Inc., Berkeley, California). It was injected iv into 14 New Zealand White rabbits (2 to 3 kg) in a dose of 0.5 μCi/g of body weight. On the following day, 7×10^4 viable BCG (Phipps strain; opacity: 0.036) (2, 3) were injected intradermally into eight different sites to establish primary infections in each of seven rabbits (Experiment I). Similar injections of BCG were also given to reinfect the other seven rabbits of the group which had received 4×10^6 viable BCG (opacity: 0.400) intradermally 4 weeks previously (Experiment II). The resulting BCG lesions were measured with calipers (2) and biopsied (3) when they were 4, 7, and 14 days of age.

On Days 0, 1, 4, 7, 11, and 14 after the ^{3}HT injection, a single dose of 0.1 ml of a 1:10 dilution of Old Tuberculin (OT) (Eli Lilly Co., Indianapolis, Indiana) was injected into each of these rabbits intradermally as an MN trap (4). The resulting tuberculin reactions (albeit nonspecific at first with primary infection) were measured and then biopsied 1 day after onset, i.e., 1 day after the OT injection (see Discussion).

Tissue sections were prepared from the biopsies and autoradiographed according to methods previously described (5). The percentage of ^{3}HT-labeled MN and their ^{3}HT grain counts[5] were then determined microscopically. The MN were evaluated in the "active" part of the BCG lesion, where they

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[4] Cells of the macrophage series cannot always be distinguished from cells of the lymphocyte series in blood or in tissue sections of inflammatory sites. The term mononuclear cells (MN) is used here to represent macrophages, although the population of cells evaluated as macrophages also contains lymphocytes. In tissue sections the MN evaluated are mainly cells of the macrophage series (1). In blood smears MN refers to an indeterminate group mostly comprised of monocytes and medium-size lymphocytes; the proportion of each is unknown.

[5] The terms "^{3}HT grains" and "^{3}HT grain count" are used for convenience in this report. In reality, the grains are silver grains in the photographic emulsion covering cells in the autoradiograph preparation. Silver is precipitated because of the radioactivity of the tritiated thymidine incorporated into the DNA of the cell.

became β-galactosidase positive and the bacilli or their products were present (see 1, 4, 5). This was the more centrally located nonnecrotic part where the bacilli were being destroyed.

Results. BCG lesions and tuberculin reactions. The sizes of the dermal BCG lesions, their necrotic centers, and the 48-hr tuberculin reactions during Experiments I and II have been already published in Table I of ref. 1. The primary lesions (Experiment I) reached a peak of about 80 mm^3 in 2 to 3 weeks. They had distinct necrotic centers at 2 weeks (1) when the rabbits became unequivocally positive to tuberculin. The lesions of reinfection (Experiment II) reached a peak of about 60 mm^3 in 1 to 2 weeks and began to heal sooner than the primary lesions. They had distinct necrotic centers by 4 days (1) as the reinfected rabbits were tuberculin positive before reinfection. (The 48-hr tuberculin reactions (1) were similar to the 24-hr tuberculin reactions listed in Table I).

In general, during the growth of the BCG lesion more cells enter than die or leave, and during the regression of the lesion fewer cells enter than die or leave. The amount of necrosis roughly reflects cell death. Each of these generalities, however, is influenced by many factors and therefore is only a very rough approximation. Most of the cells in these lesions are MN (macrophages and lymphocytes).

Blood leukocyte counts. On Days 1, 2, 5, 8, 12, and 15 after the ^{3}HT injection, the total blood leukocyte counts averaged 7450 ± 300 cells per mm^3 for the seven rabbits with primary BCG infection, and 8740 ± 410 for the seven rabbits reinfected with BCG ($P = 0.016$). In spite of this P value, these results may not be significant, as blood counts on rabbits seem to vary this much without apparent cause (unpublished observations from our laboratory). No consistent variations were found among the total blood leukocyte counts taken on the days listed in Table I.

Percentage of cells labeled with ^{3}HT. The MN in the tuberculin traps and the MN in the BCG lesions showed the highest percentage of labeling during the first 5 days after the ^{3}HT pulse (Table I and Fig. 1). Then the percentage of MN labeled in the traps declined so that 1% or less was present 12 days after the pulse.

In the tuberculin traps of the reinfected rabbits, the percentage of ^{3}HT-labeled MN was about twice the percentage in the traps of the primary infected rabbits. From the start of the experiment, the reinfected rabbits had larger traps, because they were sen-

TABLE I. PERCENTAGE OF MN LABELED WITH ^{3}HT AND THEIR AVERAGE GRAIN COUNTS IN DERMAL TUBERCULIN TRAPS AND BCG LESIONS OF EXPERIMENTS I AND II.[a]

Time after ^{3}HT-pulse biopsy was taken (Days)	Size of OT trap[b]		Percentage of ^{3}HT-labeled MN			Average grain counts on ^{3}HT-labeled MN		
	Primary	Reinfection	Primary[c] A	Reinfection[c] B	P values, A vs B	Primary[c] C	Reinfection[c] D	P values, C vs D
In OT traps								
1	±	++	13.7 ± 1.7	25.7 ± 4.1	0.010	15.7 ± 1.0	16.3 ± 0.9	
2	±	++	9.9 ± 1.3	20.2 ± 3.4	0.010	14.1 ± 0.8	15.0 ± 0.9	
5	+	++	10.3 ± 1.7[d]	16.5 ± 3.2[e]	0.056	9.1 ± 0.6	9.0 ± 0.6	
8	+	+++	2.4 ± 1.1	5.7 ± 1.8	0.075	8.7 ± 0.4	9.5 ± 0.6	
12	+++	+++	0.2 ± 0.1	1.0 ± 0.3	0.006	7.2 ± 0.7	7.1 ± 0.4	
15	+++	+++	0.3 ± 0.03	1.0 ± 0.2	0.002	7.1 ± 2.0	7.1 ± 0.4	
In BCG lesions								
5	+	++	32.6 ± 2.5[d]	28.5 ± 2.7[e]	–	12.6 ± 0.7	9.5 ± 0.5	0.002
8	+	+++	20.9 ± 4.1	9.6 ± 1.1	0.010	9.6 ± 0.5	7.6 ± 0.5	0.008
15	+++	+++	12.9 ± 4.5	6.7 ± 1.3	0.110	–	–	–

[a] In these experiments a single pulse of ^{3}HT (0.5 μCi/g of body weight; sp act 20 Ci/mmole) was injected iv into rabbits 1 day before dermal primary or reinfection BCG lesions were begun. There were seven rabbits in each group. Only cells with ^{3}HT grain counts of five and above were considered ^{3}HT-labeled. The tuberculin traps were measured and biopsied when they were 1-day-old. The means and their standard errors are given. The P values were determined by the Student's t test.

[b] Size of 1-day tuberculin (OT) reactions: ±, 3-20 mm^3; +, 20-50 mm^3; ++, 50-300 mm^3; +++, 300-600 mm^3. Reference (1) lists the 2-day tuberculin reactions.

[c] "Primary" and "reinfection" stand for rabbits bearing primary BCG lesions and BCG lesions of reinfection, respectively.

[d] P value: 10.3 ± 1.7 vs 32.6 ± 2.5 was 0.001.

[e] P value: 16.5 ± 3.2 vs 28.5 ± 2.7 was 0.007.

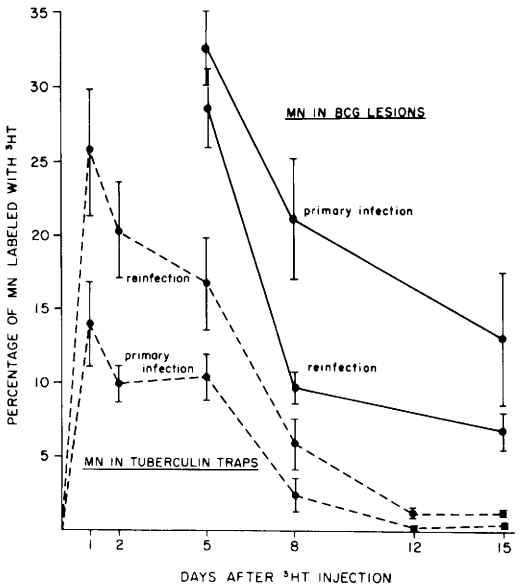

FIG. 1. Percentage of MN labeled with ^{3}HT in the BCG lesions and tuberculin traps of rabbits in Experiments I and II. ^{3}HT was injected iv 1 day before the primary lesions and those of reinfection were begun. Cells considered labeled contained five or more ^{3}HT grains.

sitive to tuberculin. In fact, rabbits with primary infection showed hardly any cell infiltrate during the first 2 days (Table I).

In contrast, in 7- and 14-day BCG lesions of reinfection, the percentage of MN labeled with ^{3}HT was about half of the percentage in corresponding primary BCG lesions. The BCG lesions usually contained more ^{3}HT-labeled MN than the 1-day tuberculin traps (see Discussion).

^{3}HT grain counts. Rabbits with primary BCG infection and those with reinfection showed identical grain counts in the ^{3}HT-labeled MN in their tuberculin traps. These counts decreased to about half during the first 5 to 8 days and showed little decrease subsequently (Table I).

At 5 and 8 days after the ^{3}HT pulse, MN in the primary BCG lesions showed slightly higher grain counts than MN in lesions of reinfection (Table I), probably for the following reason. ^{3}HT-labeled MN with high grain counts entered the lesions 2 days after the pulse, but the slower turnover in primary lesions (see Discussion) enabled such cells to remain longer.

Discussion. The term "trap" is used in this report to refer to the trapping of blood MN (monocytes and some lymphocytes) and other leukocytes in a 24-hr inflammatory site produced by an intradermal injection of tuberculin. We have no information on how many "trapped" MN remain in the sites: some drain in the lymphatics to the local lymph nodes (6). The "trap" therefore is merely a sampling device for MN that emigrate from the blood into an inflammatory site and remain there for short or long periods of time. The traps are selective in that they only sample the population of circulating leukocytes chemotactically attracted into an inflammatory site (7). During the first week after the iv ^{3}HT pulse, ^{3}HT-labeled monocytes (8, 9) and ^{3}HT-labeled short-lived lymphocytes (10, 11) enter sites of inflammation. However, by 2 weeks after the ^{3}HT pulse, some of the ^{3}HT-labeled (previously short-lived) lymphocytes in the circulation had become long-lived ^{3}HT-labeled lymphocytes, which do not readily enter sites of inflammation (10, 12).

The 1-day tuberculin reactions probably trap an MN population rather similar to the population trapped in the actual BCG lesions. Tuberculin and BCG have many antigens in common, and in the sensitive host both cause typical delayed hypersensitivity (DH) reactions. Monocytes and short-lived lymphocytes seem to accumulate nonspecifically in DH reactions and also in other types of inflammation (see Discussion in refs. 11 and 12). We feel therefore that the 1-day tuberculin reaction would collect an MN population similar to that entering BCG lesions during the same time period.

More mononuclear cells (MN) entered BCG lesions of reinfection than lesions of primary infection, because the lesions of reinfection increased in size more rapidly (see above and ref. 1), and the cell densities in each were similar (2). More MN left the lesions of reinfection than left the primary lesions (via the lymphatics or by dying *in situ*), because a decrease in the ^{3}HT-labeled MN present occurred more rapidly and caseous centers appeared sooner. In other words, there was a higher rate of MN turnover in the lesions of reinfection. This result is undoubtedly due to the preexistence of

delayed hypersensitivity (DH) in these animals. DH reactions are characterized by MN infiltration, increased lymphocyte flow and sometimes cell necrosis. Chemotactic substances, including lymphokines (7), are probably responsible for the former.

Reinfected rabbits contained a higher percentage of ^{3}HT-labeled MN in their 1-day tuberculin traps than rabbits with primary infection. This was especially true early in the course of the infection when the inflammation from the tuberculin causing the trap was much more intense in the previously sensitized rabbits. It is known that young, recently dividing macrophages and lymphocytes preferentially accumulate in sites of inflammation (8–11). Thus, one would expect sites of intense inflammation to trap more of these young cells than sites of weak inflammation (12).

Two weeks after the ^{3}HT pulse, when 1-day traps showed that very few ^{3}HT-labeled MN could be trapped from the circulation, a relatively high percentage of ^{3}HT-labeled MN was found in the BCG lesions. This result can be explained by the fact that these cells accumulated in the BCG lesions over a period of several days, whereas such cells accumulated in the traps for only 1 day.

Summary. Rabbits were infected or reinfected intradermally in multiple sites with BCG. One day before the BCG infection they were injected iv with a single pulse of tritiated thymidine (^{3}HT). At various times thereafter, the BCG lesions were biopsied and evaluated for ^{3}HT-labeled mononuclear cells (MN), which are mostly macrophages but include some lymphocytes. Periodically Old Tuberculin (OT) was injected intradermally, creating MN traps, which were biopsied and evaluated 1 day after their onset. These traps monitor the population of ^{3}HT-labeled MN in the blood stream that readily enters sites of inflammation.

During the first 5 days after the ^{3}HT pulse, a higher percentage of labeled MN accumulated in tuberculin traps on rabbits reinfected with BCG than in traps on rabbits with primary infection. The high percentage of labeled MN in the former was probably due to the more intense inflammation caused by preexisting tuberculin hypersensitivity. By 8 days after the ^{3}HT pulse, a lower percentage of labeled MN had accumulated in BCG lesions of reinfection than in primary BCG lesions. Evidently the presence of delayed hypersensitivity increased the turnover of MN in tuberculous lesions. By 15 days after the ^{3}HT pulse, the percentage of ^{3}HT-labeled MN was much higher in BCG lesions than in 1-day traps, suggesting that such MN had accumulated in the lesions over a period of several days.

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