ISOPROTERENOL-INDUCED LYMPHOCYTE-DEPENDENT SALIVARY GLAND HYPERPLASIA IN MICE: A POSSIBLE ANALOG OF SYNGENEIC MIXED LYMPHOCYTE CULTURE IN VIVO

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KEY WORDS: T lymphocytes; salivary glands; syngeneic mixed lymphocyte culture

The writer showed previously that T lymphocytes, which can both trigger hyperplasia and reduce its intensity, participate in the hyperplastic response of rodent salivary glands to isoproterenol (IP) [2]. The lymphocytic response under these circumstances may be estimated quantitatively as the degree of blast formation in the spleen 4-8 h (stimulating cells) and 22-26 h (inhibiting cells) after injection of IP. On the basis of data reflecting the role of lymphocytes in widely different processes in which cell division of both immune and somatic cells takes place [1, 2, 4, 8], and of a theoretical analysis of self-organization of cellular proliferation at the cell population level, the writers postulated that a proportion of lymphocytes takes place, not in the immune response, but in the regulation of cell division of somatic tissues [3]. Analysis of the properties of different lymphocyte subpopulations showed that the most likely candidates for the role of these cells in mammals are lymphocytes, participating in what has been called syngeneic mixed cell culture [3]. Monoclonal antibodies have been obtained against these lymphocytes, abolishing the reaction of syngeneic mixed cell culture, but a typical immune response such as allogeneic mixed lymphocyte culture was unchanged under these circumstances [7, 9].

In the investigation described below the properties of T cells, participating in the hyperplastic response of the salivary glands to IP, and the possibility of regarding this response as an analog of syngeneic mixed lymphocyte culture in vivo were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 250 male BALB/c mice weighing 18-20 g, in which a hyperplastic response of the salivary glands was induced by intraperitoneal injection of 5 mg IP. The animals were killed 4 and 24 h later and a fraction rich in blast cells was obtained from their spleen by centrifugation in a Ficoll—Verografin density gradient, with a density of 1.07 [10]. To assess the proliferative activity of these cells, 10^6 cells were cultured in medium 199 with 5 μCi of ^{3}H-thymidine for 2.5 h at 37°C and incorporation of the label into the acid-insoluble fraction (DNA) was determined on a liquid scintillation counter. The phenotype of the proliferating cells was estimated on the basis of the action of antiserum against Thy-1 antigen in the presence of complement, the antiserum having been obtained by immunizing rabbits with mouse brain homogenate [6], and also by the use of monoclonal antibodies against Ly-antigens. Monoclonal RTα antibodies against Ly-1 antigen was used in conjunction with anti-immunoglobulin MAR-18.5 monoclonal antibodies (Japan), and also monoclonal anti-Ly-2 antibodies (obtained and tested by Dr. Med. Sci. V. D. Brondz) were used.

Conditions of culture of the cells thus obtained were studied. Cells treated and untreated with anti-Ly-sera were cultured for 1-3 days in medium 199 with 100 μg gentamycin, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and 40% of preparations containing interleukin-1 (IL-1) or interleukin-2 (IL-2), the intensity of proliferation being estimated from the uptake of ^{3}H-thymidine into the cultures during 6 h of culture daily. Preparations containing IL-1 and IL-2 were obtained by treating adherent cells of peritoneal washings of rats with *E. coli* lipopolysaccharide and mouse splenocytes with concanavalin A, respectively [5, 11]. Supernatants of cells activated by these agents were used in a final concentration of 40%.

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TABLE 1. Phenotype of Proliferating Splenic Blast Cells of Mice Receiving Injection of IP

Type of cells	Intensity of uptake of label (in cpm/10^6 cells) during treatment with antisera and complement			
	without treatment (intact cells)	anti-Thy-1	anti-Ly-1	anti-Ly-2
First peak — helper cells	2371±101	500±45**	869±20**	2020±118
Second peak — suppressor cells	3864±793	796±90**	2316±73	1412±145*

Legend. Uptake of label by intact spleen cells was 489 ± 41 cpm/10^6 cells, after treatment with anti-Thy-1-serum 334 ± 10 cpm/10^6 cells. *p < 0.1, **p < 0.01 compared with intact cells.

TABLE 2. Effect of Preparations Containing IL-1 and IL-2 on Proliferation of Helper-Type Cells from the Blast Fraction of the Spleen, Treated with Anti-Ly-Sera, during IP-Induced Hyperplasia of the Salivary Glands in Mice (3rd day of culture)

Type of cells	Uptake of ^{3}H-thymidine (in cpm/10^6 cells) under the influence of		
	nothing added	IL-1	IL-2
Not treated with antisera	1002±64	3067±384*	7019±541**
Ly-1-positive	815±78	2387±53**	3025±263*
Ly-2-positive	1326±112	612±125*	5144±1539*

Legend. *p < 0.01, **p < 0.001 compared with cells without addition of interleukins.

The tests were done in triplicate. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Proliferating blast cells of the first peak which, as was shown previously, possessed stimulating activity in the hyperplastic response of mouse salivary glands [2], consisted mainly of Thy-1, Ly-1-positive cells, whereas cells of the second peak of blast formation in the spleen, exhibiting inhibitory activity in syngeneic transfer, consisted of Thy-1, Ly-2-positive cells (Table 1).

Spontaneous proliferation of both types of cells continued for only the first day of culture in serum-free medium (Fig. 1) but was maintained throughout the period of observation after addition of IL-1 for cells of the first peak or IL-2 for cells of the first and second peaks (Figs. 1 and 2).

The use of anti-Ly-sera showed that cells of the first peak maintain proliferative activity in the presence of IL-1 only in the additional presence of both Ly-1-positive and Ly-2-positive cells, whereas IL-2 activated proliferation of both types of cells (Table 2).

The results can be interpreted well from the point of view of the properties of T lymphocytes participating in syngeneic mixed lymphocyte culture [7, 9]: Ly-1-positive cells,

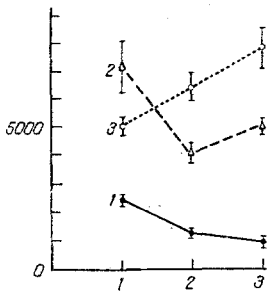

Fig. 1

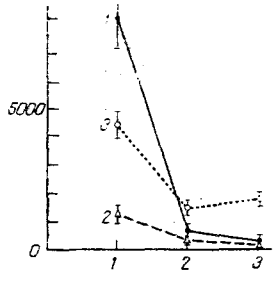

Fig. 2

Fig. 1. Culture of cells of stimulating fraction obtained 4 h after injection of IP into mice. Abscissa, duration of culture (in days); ordinate, uptake of ^{3}H-thymidine (in cpm/10^6 cells). 1) Nothing added; 2) addition of 40% of preparation containing IL-1; 3) addition of 40% of preparation containing IL-2.

Fig. 2. Culture of cells of inhibiting fraction obtained 24 h after injection of IP into mice. Legend as to Fig. 1.

activated in the course of these processes by IL-1, secrete IL-2, which itself maintains proliferation of Ly-1- and Ly-2-positive cells of both helper and suppressor types.

On the basis of views developed along these lines, IP-induced lymphocyte-dependent salivary gland hyperplasia in mice can be interpreted as an analog of syngeneic mixed lymphocyte culture in vivo. The G_0/G_1-transition, stimulated in gland cells by IP, leads to definite changes in these cells, which are transmitted by Ly-1-positive T helper cells with the participation of IL-1 (evidently with the participation of A cells), which triggers proliferation of Ly-1-positive T helper cells and secretion by them of stimulating factors, including IL-2, which maintains proliferation of T cells of both helper and suppressor types. As was shown previously [2], Ly-1-positive T helper cells are essential for development of the hyperplastic response of the submandibular salivary glands in mice, whereas the accumulation of T suppressor cells evidently arrests the process.

It can be tentatively suggested that reactions of syngeneic mixed lymphocyte culture in vitro, recently described, reflects important processes of regulation of cell division in the multicellular organism in vivo.

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INTERSPECIFIC SIMILARITY IN THE TRAJECTORY OF REACTIVE LEUKOCYTOSIS

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KEY WORDS: uncomplicated postoperative period; reactive leukocytosis

The writers suggested previously that fluctuations of the leukocyte count during reactive leukocytosis in dogs are not random in character but obey a definite rule [6]. On the basis of data in the literature [8] it was postulated that this rule also extends to reactive leukocytosis in man.

The object of the investigation described below was to test this hypothesis, by studying the peripheral blood leukocyte count in patients in the course of an uncomplicated postoperative period.

EXPERIMENTAL METHOD

Observations were made on 60 patients (26 men and 34 women) aged from 18 to 48 years after appendectomy, who volunteered to take part in the clinical investigation. An appendix abscess was present in 45 patients (75%), catarrhal appendicitis in 15 (25%). All patients were admitted to hospital 2-48 h after the onset of the illness. The postoperative period

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