CYTOLOGICAL CHARACTERISTICS OF THE MOUSE SPLEEN DURING INDUCTION OF TOLERANCE AND SUBSEQUENT INJECTION OF THE CORRESPONDING ANTIGEN

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Immunologic tolerance was induced in mice by successive injections of sheep's red cells and cyclophosphamide. During the induction of tolerance an initial increase in the number of plasma and blast cells in the spleen was followed by pancytopenia. Later, hyperregeneration of the myeloid and erythroid cells was observed, and this was followed by restoration of the normal cell composition of the spleen. Similar changes, with the exception of the initial increase in the number of plasma and blast cells, were observed in mice receiving cyclophosphamide only. Subsequent injection of antigen in the tolerant animals did not induce a plasma-cell response by contrast both with the control animals and with animals receiving cyclophosphamide only.

KEY WORDS: immunologic tolerance; cyclophosphamide; lymphoid tissue; plasma cell response.

Lymphoid tissue responds to injection of an antigen not only by the formation on antibody-forming cells and memory cells but also by other reactions of only relative specificity. These include an increase in the number of blast and plasma cells (including cells producing "nonspecific" γ-globulins), the formation of secondary follicles, and so on. Experimental data on the presence or absence of such reactions in tolerant animals are comparatively few in number and often contradictory in nature [1, 9, 11-13, 18, 19]. The investigation of this problem is of twofold importance: 1) to determine the complete characteristics of the various forms of tolerance and their possible differences; 2) to assess relations between the specific and "nonspecific" forms of immune response.

With these considerations in mind, a parallel study was undertaken of the cytological features and antibody-forming function of the spleen in mice in which tolerance was induced by consecutive injections of sheep's red cells and the immunodepressant cyclophosphamide. This model was first subjected to a detailed study by various immunologic and immunomorphologic methods [2, 5-7].

EXPERIMENTAL METHOD

Experiments were carried out on adult male F_1(CBA × C57BL/6) hybrid mice and noninbred albino mice weighing 18-25 g. Tolerance was produced by injecting sheep's red cells (SRBC) intraperitoneally in a dose of $6 \cdot 10^9$, followed 2 days later by cyclophosphamide (CP) in a dose of 200 mg/kg. Control animals received CP alone, antigen alone, or neither. The animals were killed after various periods and squash preparations obtained of the spleen, fixed with methyl alcohol, and stained by the May-Gruenwald method and counterstained with azure-eosin. The cytogram of the spleen was established by identifying 3000-5000 cells. The absolute number of cells of each line was counted for the whole spleen. Four analogous experiments were carried out, in which an average of 20 mice were used at each time. Some of the animals were immunized intravenously with a test dose of $5 \cdot 10^8$ SRBC 21 days after receiving the injection of CP. The

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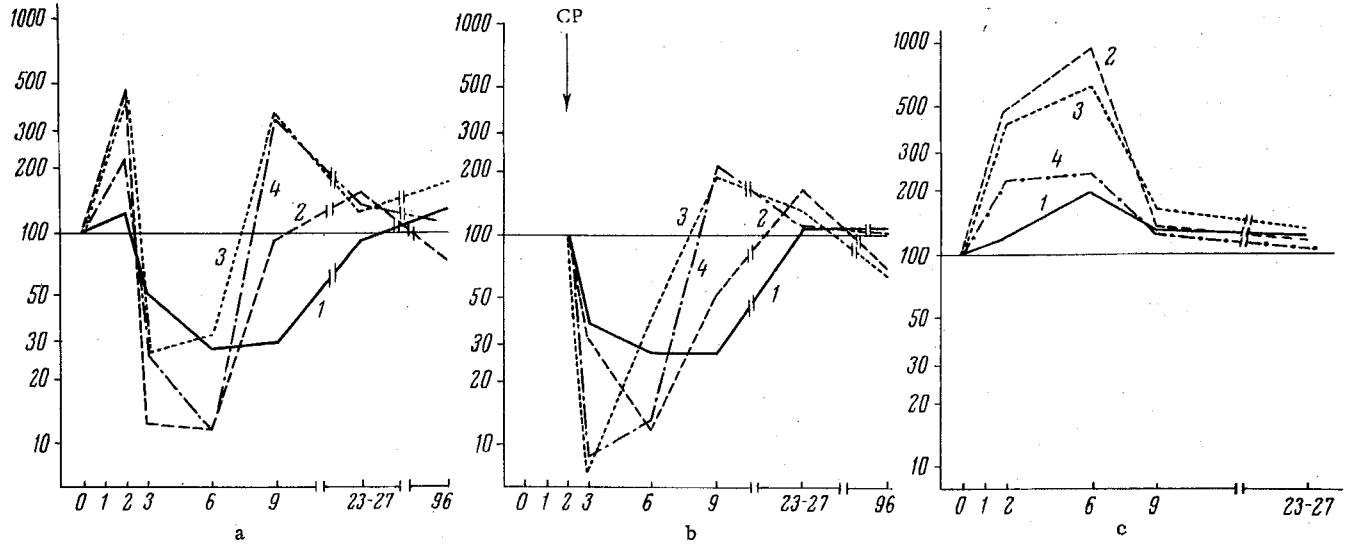

Fig. 1. Number of nucleated cells in mouse spleen after injection of $6 \cdot 10^9$ SRBC and CP (a), CP (b), and $6 \cdot 10^9$ SRBC (c): 1) lymphocytes; 2) plasma cells; 3) blast cells; 4) myeloid and erythroid cells. Abscissa, days after injection of SRBC and CP; ordinate, number of cells (in % of control).

TABLE 1. Immunomorphological Changes in Spleen of Tolerant Animals after Test Injection of Antigen

Animals	No. of animals*	Test injec. of antigen	Plasma cells ($1 \cdot 10^6$)	Blast cells (1×10^6)	Lymphocytes (1×10^6)	Myeloid-erythroid cells	Mitoses (1×10^6)	AFCs (1×10^3)
Tolerant	26/18	—	3,4±0,5	1,4±0,2	140±11	43,4±8,4	2,2±0,7	$\leqslant 0,2 \overset{\times}{:} 1,3$ †
	19/19	+	3,3±0,4	1,8±0,5	145±8	27,5±4,4	1,7±0,3	$1,3 \overset{\times}{:} 1,6$ †
Receiving CP only	18/6	—	3,4±0,5	1,3±0,2	138±9	39,4±4,5	2,2±0,4	$\leqslant 0,1 \overset{\times}{:} 1,1$
	16/16	+	6,1±1,2	2,8±0,6	172±11	49,4±7,3	3,0±0,7	$41,4 \overset{\times}{:} 1,4$
Intact	22/11	—	2,2±0,3	1,1±0,1	147±16	32,5±4,1	1,8±0,4	$\leqslant 0,1 \overset{\times}{:} 1,1$
	18/18	+	5,8±0,8	2,3±0,5	184±12	30,7±4,6	2,6±0,5	$90,6 \overset{\times}{:} 1,2$

*Numerator gives number of animals used in morphological experiments; denominator gives number of animals investigated by Jerne's test.

†Values differing by a statistically significant degree are braced together.

number of antibody-forming cells (AFCs) in the spleen of the mice was determined 4 days later by the method of Jerne and Nordin [14] and the cytogram of the spleen was studied.

EXPERIMENTAL RESULTS

The cellular composition of the spleen of the intact mice (control) was as follows (mean values, millions): lymphocytes 118.4, erythroid cells 42.3, myeloid cells 15.3, plasma cells 1.8, blast cells 0.9, dividing cells 1.5.

The cellular composition of the spleen of mice receiving $6 \cdot 10^9$ SRBC, or CP, or both is shown in Fig. 1 as percentages of the control level.

Injection of a large dose of antigen induced the changes usually associated with antigenic stimulation: a marked increase initially of blast cells and later of plasma cells. By the 6th day the number of lymphocytes also was increased; changes in the myeloid and erythroid cells were not statistically significant. By the 9th day after antigenic stimulation the cell composition of the spleen was basically back to normal (Fig. 1c).

Different changes were induced by CP (Fig. 1b). Initially (after 1-4 days) pancytopenia was observed but this was soon followed by compensatory hyperplasia of the myeloid and erythroid cells; lymphopoiesis, by contrast, recovered much later. At later periods of observation the cell composition of the spleen gradually returned to normal.

The number of plasma cells at first decreased sharply, but later it fluctuated within wide limits. The number of blast cells corresponded in general to the dynamics of the erythro-myeloid series. On the whole, the dynamics of the changes in the cell composition of the spleen after injection of CP corresponded to the data recorded previously [8].

Cell changes induced by the combined injection of a large dose of antigen and CP (tolerogenic treatment; Fig. 1a) were more complex in character. On the 2nd day of the experiment the number of blast and plasma cells was considerably increased. After the injection of CP the cell composition of the spleen changed sharply: pancytopenia developed (3rd-6th day), followed by lymphomyeloid hyperplasia (9th day) and, finally, restoration of the normal cell composition of the spleen (23rd-91st day).

Administration of the immunodepressant thus completely arrested the commencing response to the antigen. Rosette-forming cells also disappeared [2].

By the time of the test injection of antigen the cell composition of the spleen of the tolerant mice was indistinguishable from normal (Fig. 1a; Table 1). Only the number of plasma cells showed a small increase, although this was observed also in animals receiving CP alone. However, their response to the test injection of antigen was substantially different (Table 1).

Both the intact mice and mice treated previously with CP responded to antigen by an increase in the number of plasma and blast cells (by 2-3 times), by a small increase in the number of lymphocytes, and by a well-marked specific response – the formation of numerous AFCs. Conversely, in the tolerant mice no statistically significant changes were found in the number of plasma or blast cells and lymphocytes; only a very few AFCs were formed (30-70 times fewer than in the control). The formation of rosette-forming cells after injection of antigen is also sharply inhibited in tolerant animals [2].

The absence of a plasma-cell response in the tolerant mice in the present experiments agrees with data [11-13, 19] obtained with a different model of tolerance (high-zone tolerance to soluble proteins). Only some plasma cells formed in the course of an immune response are known to synthesize specific antibodies, which can be determined by the usual methods. A similar situation occurred also in the present experiments: the number of plasma cells in the control groups increased from $(2-3) \cdot 10^6$ to $(5-6) \cdot 10^6$, whereas the number of plaque-forming cells did not exceed $9 \cdot 10^4$. The method used took into account only 19S-AFCs, but at the times of investigation (4 days after the test injection of antigen) virtually no 7S-antibodies were synthesized. The 30-fold difference in the number of newly formed plasma cells and plaque-forming cells observed in the control animals thus reflected the "nonspecific" component of the immune response. Impairment of this "nonspecific" component in tolerant animals is of interest from the theoretical point of view. Experiments have shown [3, 10, 15, 16] that the function of the T-cells is primarily affected during tolerance to SRBC and protein antigens. It can therefore be postulated that activation of the corresponding clone of T-cells by the antigen and interaction between these T-cells and "nonspecific" clones of B-cells

is an essential preliminary to the plasma-cell response. So far as the dynamics of the cytological changes during the induction of tolerance is concerned, this agrees well with the view that death of cell clones involved in proliferation is the leading mechanism of "drug-induced" tolerance [2, 4, 6, 7, 17].

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