

In Vitro Mitotic Activity of Unsensitized Lymphocytes in the Presence of Antigen and Sensitized Lymphocytes¹ (34649)

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A population of lymphoid cells multiplies *in vitro* when cultured with a given antigen if the cells derive from an animal that has previously been immunized with the same antigen. This proliferative response has been associated with both delayed hypersensitivity (1) and humoral states of immunity (2). The proportion of cells which responds to an antigen has been assessed by measuring the number of cells synthesizing DNA, the number of metaphases collected in a specified interval, and the number of cells exhibiting morphological transformation. Values for the number of cells responding to antigen stimulation vary from 0.1 to 40%; these values appear to be high for a strictly clonal organization of immunological memory. One possible explanation of the high proportion of responding cells that would be compatible with a clonal organization of the immunological memory is the recruitment of unsensitized cells by sensitized clonally derived cells in the presence of antigen. It has been shown that the culture medium and extracts of antigen-stimulated lymphoid cells are capable of inducing proliferation in a population of separately cultured unsensitized cells (3), but the contribution of this recruitment phenomenon to the total response of a sensitized cell population—a critical point for the explanation of the high numbers of immunologically reactive cells—is not known. By culturing lymphoid cells from male rats with delayed hypersensitivity to PPD together with an equal number of lymphoid cells from syngeneic unsensitized female rats and

PPD, such recruitment could be demonstrated, and the proportion of recruited cells measured, by the identification of a significant number of XX karyotypes in the population of responding cells.

One to five million thoracic duct lymphoid cells from PPD-sensitive (4) "Fischer 344" male rats (A. R. Schmidt Co., Inc., 2826 Latham Drive, Madison, Wis. 53713) were washed, mixed with an equal number of thoracic duct cells from unsensitized "Fischer 344" female rats, and then cultured with 5–10 $\mu\text{g}/\text{ml}$ of PPD (Parke-Davis) in 5 ml of Dulbecco-Vogt medium (5) containing 10% fresh rat serum, penicillin (100 U/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$), at 37° for a total of 72 hr. After 68 hr of incubation, the metaphases were collected by adding colcemide (0.02–0.04 $\mu\text{g}/\text{ml}$) for the last 3–4 hr of incubation. The metaphases were counted and karyotyped according to the normal "Fischer 344" rat karyotypes illustrated in Fig. 1. Appropriate control experiments were run simultaneously. The results of the experiments are summarized in Table I. When the two types of cells were cultured separately with PPD, the population of sensitized cells (XY) contained a significantly higher proportion of metaphases than the unsensitized population (XX). In the 20 cultures in which sensitized and unsensitized cells were mixed together with PPD, 730 metaphases were found. Of these, only 18 permitted the unambiguous distinction of male and female karyotypes. Eight of the karyotypes were XX and therefore unsensitized, and 10 were XY and therefore from sensitized animals. The probability is less than 1/20 that the 18 metaphases were drawn from a total population of metaphases that had less than 21% or more than 67% female karyotypes. The very low mitotic

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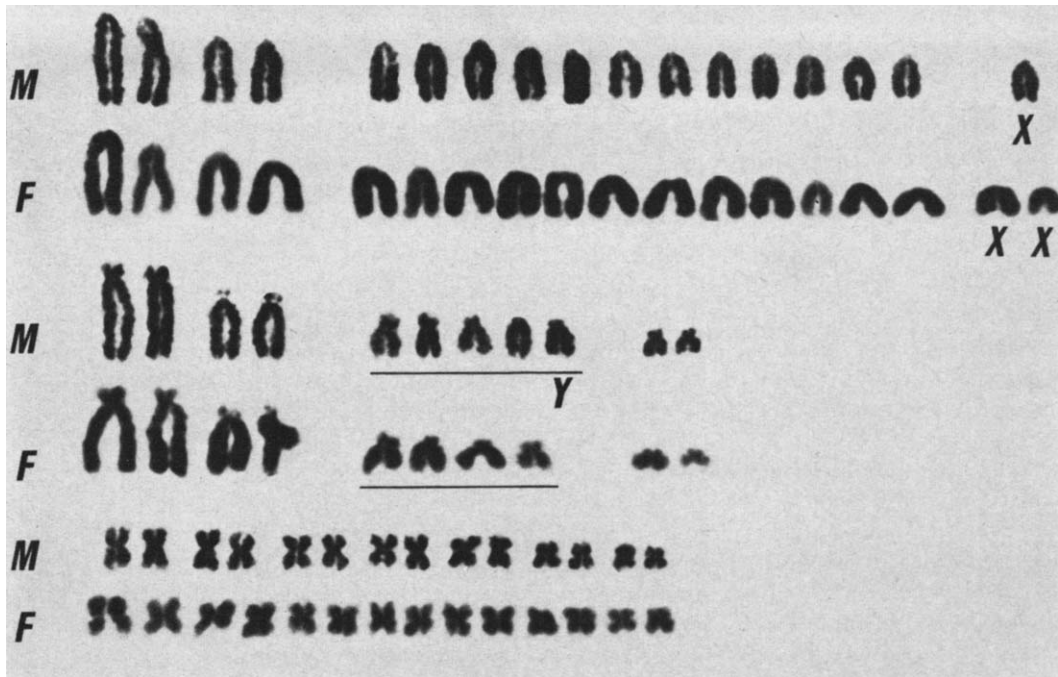


FIG. 1. Karyotypes of the male (M) and female (F) "Fischer 344" inbred rats from thoracic duct lymphoid cells stimulated with phytohemagglutinin. The X chromosomes belong to the group of medium size acrocentrics. The Y chromosome is the fifth member of the small acrocentrics with satellites.

activity in mixed cultures (XX cells + XY cells) without PPD provides assurance that the replication of the cultured cells cannot be attributed to antigenic differences between male and female lymphocytes.

Since it is evident that unsensitized cells are capable of responding to antigen in the presence of cells from a sensitized animal, it is possible that a large proportion of the responding cells in the sensitized population

are not specifically committed to respond to PPD but are recruited to proliferate by a comparatively small group of committed cells. If the value of 44% is taken as the best estimate of the ratio $XX/(XX + XY)$ found in this experiment, and the responsiveness of uncommitted cells from the sensitized is equal to that from the unsensitized animals, then it may be calculated that only 12% of the responding cells from the sensi-

TABLE I. Mitotic Activity of Thoracic Duct Lymphoid Cells.

XY cells are from sensitized animals; XX cells from unsensitized animals. $XX/(XX + XY)$ is the ratio of identifiable female karyotypes to total karyotypes for which sex was assignable.

Combinations	No. of experiments performed	Total metaphases/total cells counted	Occurrence of metaphases/ 10^8 cells (means and range)	Ratio of metaphases $XX/(XX + XY)$
XY cells + PPD	7	$121/143 \times 10^8$	2.4 (0.2-8.3)	
XX cells + PPD	5	$0/244 \times 10^8$	<0.004	
XY cells + XX cells	6	$2/359 \times 10^8$	0.005	
XY cells + XX cells + PPD	20	$730/340 \times 10^8$	3.2 (0.2-18.0)	8/18
XY cells + Phytohemagglutinin	2		25.0 (10-40)	1/17
XX cells + Phytohemagglutinin	2		13.5 (6-21)	12/12

tized animals are specifically committed.³ If the true ratio $XX/(XX + XY)$ is closer to 50%, then the number of specifically committed cells dividing in response to antigen stimulation may be very small indeed. Obviously, the present data do not permit a precise determination of the size of the committed population but the results do demonstrate that the number of cells undergoing mitosis after antigenic stimulation is not a reliable indicator of the number of specifically committed cells in a given population of lymphoid cells, and suggest that they are very scarce indeed. There are other examples of the participation of unsensitized cells in manifestations of delayed hypersensitivity (6) and it has been suggested recently that the humoral immune response may also entail stimulation of uncommitted cells to make antibody (7) so that recruitment may prove to be a general feature of the immune response.

Summary. Thoracic duct lymphocytes from male rats with delayed sensitivity to PPD were cultured together with lymphocytes from syngeneic unsensitized female rats. The addition of PPD to the cultures caused a significant increase in mitotic activity of roughly equal magnitude in both sensitized and unsensitized cells. Assuming a clonal organization of the immunological memory, these observations make possible that most cells dividing in response to antigen stimulation are not committed cells [*i.e.*, members of the stimulated clone] but "recruited" uncommitted cells, thus lowering the concentration of the clone in the population of lymphoid cells.

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³ Since total responding cells (rc) = [committed cells from sensitized animals (cc-S) + uncommitted cells from sensitized animals (uc-S) + uncommitted cells from unsensitized animals (uc-U)], and (uc-S) = (uc-U) = 44%, then, when rc = 100 = cc-S + (2 × 44), cc-S = 12.

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