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Lines of T Lymphocytes Induce or Vaccinate Against Autoimmune Arthritis

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Abstract. *The pathophysiology of autoimmune arthritis was studied by selecting and isolating lines of effector T lymphocytes from rats administered an arthritogenic dose of* Mycobacterium tuberculosis *in complete Freund's adjuvant to induce adjuvant arthritis. Irradiated rats were intravenously inoculated with a cell line characterized by proliferative reactivity to* Mycobacterium tuberculosis *and, to a lesser degree, to rat collagen type II. This produced arthritis in all the irradiated rats. Nonirradiated recipients failed to develop arthritis. However, such rats, and those recovering from cell-mediated arthritis, were resistant to subsequent attempts to induce adjuvant arthritis. Lines of T lymphocytes selected for responsiveness to other antigens had no effect. Therefore, a line of T lymphocytes responsive to bacteria or to collagen type II could either induce autoimmune arthritis or serve as an agent of vaccination against it.*

Rheumatoid arthritis is characterized by a chronic proliferative and inflammatory reaction in synovial membranes, producing pain, disability, and eventual destruction of joints (*1*). Although the etiology of this disease is unknown, it is thought that autoimmune processes are involved (*2*).

An animal model of arthritis that has features similar to human rheumatoid arthritis is adjuvant arthritis (AA) (*3*). Adjuvant arthritis can be induced in rats by a single intradermal injection of a suspension of killed *Mycobacterium tuberculosis* in complete Freund's adjuvant (CFA). Experimental evidence suggests that an autoimmune process involving T lymphocytes is responsible for the generation of AA. Another form of autoimmune arthritis can be induced in rats (*4*) and mice (*5*) by immunization with type II collagen, component of joint cartilage. The development of this arthritis is associated with both cell-mediated and humoral immunity to type II collagen (*6*). Immunity to type II collagen has also been detected in human rheumatoid arthritis (*7*) and in AA (*8*).

We investigated the means by which *M. tuberculosis* induces the autoimmune joint damage seen in AA and the role that reactivity to type II collagen might play in the process by isolating and propagating arthritogenic effector cells as T lymphocyte lines. This approach is based on the observation that T cell lines reactive against the basic protein of myelin can both induce and vaccinate against experimental autoimmune encephalomyelitis (*9*). Accordingly, we isolated three T cell lines. From Lewis rats immunized with CFA we selected one line (designated Z1c) for its proliferative reactivity to the purified protein derivative of *M. tuberculosis* and a second line (designated A2) for its reactivity to the whole bacterium. A third line (designated D1) was selected for its reactivity to type II collagen from Lewis rats that had been immunized with rat type II collagen in incomplete Freund's adjuvant.

Table 1 shows the proliferative responses of the three cell lines. Line A2 reacted strongly to *M. tuberculosis*, to a lesser extent to purified protein derivative, and weakly to collagen type II. Despite its low magnitude, the response of line A2 to collagen type II was similar to that of line D1, which was directly selected for reactivity to type II collagen. These relatively low proliferative responses may be the result of the physicochemical properties of collagen fibers (*10*), which could make them weak activators of T cells in vitro. Line D1 did not show any reactivity to *M. tuberculosis* or the protein derivative. Line Z1c showed reactivity to the protein derivative, and to a lesser extent to the bacterium, but not to type II collagen.

We then investigated whether line A2 can induce arthritis or be used to vaccinate against subsequent induction of active AA. Because total body irradiation before inoculation with adjuvant increases the susceptibility of rats to AA (*11*), we inoculated the A2 line into both nonirradiated and irradiated rats. Intravenous inoculation with 2×10^7 untreated A2 cells did not lead to development of arthritis in rats that had been irradiated with 200 R (Table 2). However, inoculation of the A2 cells into rats irradiated with 750 R led to polyarthritis within 6 to 12 days. This arthritis lasted for up to 3 weeks and was characterized by the inflammation and histological features of AA. Irradiation of A2 cells with 1500 R abrogated their ability to cause arthritis in irradiated rats. Control T cell lines selected for their reactivity to the protein derivative (Z1c), to ovalbumin (C1a), or to the basic protein of myelin (Z1a) did not cause arthritis.

These results indicate that specific T lymphocytes reactive against *M. tuberculosis* can induce autoimmune arthritis and that suppressor mechanisms sensitive to radiation can participate in the regulation of arthritis (*11*). Although antibodies to collagen type II may be capable of transferring arthritis to recipient rats (*12*), it is unlikely that the transferred T lymphocytes (negative for immunoglobulin markers) or the heavily irradiated recipients could have produced arthritogenic antibodies. Thus, it appears that the A2 cells themselves mediated the arthritis.

To test whether the A2 line can also vaccinate rats against active AA, we challenged rats with CFA 35 days after they had been inoculated with A2 or

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Table 1. Proliferative responses of T lymphocyte lines. Lines A2 and D1 were obtained from male Lewis rats that had been injected in each hind foot pad with 0.05 ml of CFA containing 10 mg of heat-killed *Mycobacterium tuberculosis* ($H_{37}Ra$; Difco) per milliliter (for line A2) or with 0.05 ml of rat type II collagen (donated by E. J. Miller) emulsified in equal volumes of 0.1*M* acetic acid and incomplete Freund's adjuvant (Difco) (for line D1). On day 9 the draining lymph nodes were removed and T cell lines reactive to the sensitizing antigens were generated by intermittent 3-day periods of incubation with antigen as described in (*9*), but without the density separation step. Ten micrograms of *M. tuberculosis* or rat type II collagen per milliliter were used as stimulating antigens for lines 2A and D1, respectively. Line Z1c was obtained from female Lewis rats that had been immunized with basic protein in CFA (*9*). The proliferative responses of these cells (2.5×10^4 per ml) were tested in vitro (*9*) with antigens at optimum concentrations determined by dose-response experiments. These antigens included *M. tuberculosis* (50 μg/ml), purified protein derivative (Statens Serum Institut) (25 μg/ml), rat type II collagen (50 μg/ml), conconavalin A (Miles Yeda) (2.5 μg/ml), and accessory cells in form of irradiated (1500 R) syngeneic thymus cells. The proliferative responses were measured as counts of [^{3}H]thymidine incorporated into DNA per minute or as the stimulation index (the ratio of control to test measurements) (*9*). All the cells of lines developed in this way bear specific markers of T lymphocytes detected by monoclonal antibodies (*16*). Stimulation indices are given in parentheses. Values are means ± standard deviations.

T lymphocyte line	Selecting antigen	Proliferative response (count/min × 10^{-3})				
		Control (no antigen)	*M. tuberculosis*	Collagen type II	Protein derivative	Concanavalin A
A2	*M. tuberculosis*	2.7 ± 0.3	109.9 ± 5.2 (40.7)	5.9 ± 0.7 (2.2)	51.3 ± 3.0 (19)	183.3 ± 3.1 (67.9)
D1	Type II collagen	3.6 ± 0.4	2.7 ± 0.3 (0.8)	8.6 ± 0.3 (2.4)	2.5 ± 0.5 (0.7)	25.9 ± 0.8 (7.2)
Z1c	Protein derivative	1.2 ± 0.2	25.4 ± 1.7 (21.1)	1.1 ± 0.3 (0.9)	67.6 ± 2.3 (56.3)	87.2 ± 2.1 (72.7)

other lines. Uninjected nonirradiated rats, uninjected irradiated rats, and irradiated rats that had been given the Z1c, C1a, or Z1a control lines were highly susceptible to AA induced by injection of CFA (Table 2). In contrast, rats inoculated with intact A2 cells were fully protected against subsequent induction of active AA. This protection appeared as soon as 16 days after inoculation. Injection of line A2 cells irradiated with 1500 R protected about 60 percent of nonirradiated recipients.

Since line A2 was reactive against the *M. tuberculosis* present in CFA, vaccination against AA may have been due to inhibition of the general adjuvant effect of CFA rather than to inhibition of the lymphocytes that specifically caused arthritis. We therefore investigated whether line A2 can protect rats against experimental autoimmune encephalitis induced by injecting them with the basic protein of myelin emulsified in CFA (*13*). As shown in Table 2, recipients of line A2 were fully protected against AA, but not against encephalitis. Likewise, line Z1a (reactive to the basic protein) did not protect against AA but did protect against encephalitis. These findings indicate that protection was immunologically specific and not the result of a generalized antiadjuvant action. Since protection against AA was specific for the A2 line, and since T cell lines of different antigen specificities differ by virtue of antigen receptors, it is conceivable that this protection might have resulted from immunization against the receptors of the T lymphocytes that react against self-antigens in the joints.

It has been proposed that receptor immunity regulates immune responses by suppressing or activating specific clones of lymphocytes (*14*). The fact that irradiated rats acquired resistance to active AA suggests that the mechanism responsible for vaccination might be resistant to radiation. It is also conceivable that the A2 cells persisted in the recipient rats and immunized them after their recovery from irradiation. Preliminary studies of the effects of line D1 (specific for collagen type II) indicate that this line is not capable of inducing arthritis in either nonirradiated or irradiated (750 R) rats. However, some support for a relation between AA and collagen II immunity may be derived from the finding that four of eight rats inoculated with 5×10^6

Table 2. Induction of and vaccination against AA by A2 cells. Female Lewis rats (6 to 10 weeks old) were inoculated intravenously with 2 ± 10^7 cells of T lymphocyte lines reactive to *Mycobacterium tuberculosis* (A2), purified protein derivative (Z1c), ovalbumin (C1a), or the basic protein of myelin (Z1a). The rats were injected in a nonirradiated condition or immediately after irradiation (200 or 750 R) by a ^{60}Co gamma-ray source (GB 150A, Atomic Energy of Canada) at a distance of 86.5 cm and a dose rate of 80 rads/min. Before the injections the cell lines were restimulated in vitro by incubation of 2 ± 10^5 cells per milliliter for 72 hours in the presence of irradiated (1500 rads) syngeneic accessory cells (1.5×10^7 thymocytes per milliliter) and the relevant antigens: *M. tuberculosis* (10 μg/ml), protein derivative (5 μg/ml), ovalbumin (Sigma) (20 μg/ml), or basic protein of myelin (10 μg/ml) (*9*). The cells were then collected. Some were treated by irradiation with 1500 R (at a distance of 40 cm and dose rate of 370 rads/min). The rats received intravenous injections of irradiated or nonirradiated cells into the tail vein and were examined daily and scored for clinical signs of arthritis—erythema and swelling of joints. The diagnosis was confirmed by histological examination of selected rats. Thirty-five days later, the rats were tested for susceptibility to induction of AA by intradermal injections of 0.1 ml of CFA containing *M. tuberculosis* (10 mg/ml). Several rats were inoculated in each hind foot pad with 0.05 ml of basic myelin protein in CFA to assay their susceptibility to experimental autoimmune encephalitis (*9*). The number of rats in each group is given in parentheses. N.D., not done.

Cell line	Irradiated (1500 rads)	Recipient rats irradiated	Incidence of arthritis (percent)		Incidence of encephalitis (percent) by basic protein in CFA 35 days after inoculation
			Cell-induced arthritis	AA induced by CFA 35 days after cell inoculation	
A2	No	No	0 (57)	0 (52)	100 (5)
	No	200 R	0 (9)	0 (9)	N.D.
	No	750 R	100 (12)	0 (12)	N.D.
	Yes	No	0 (9)	44 (9)	N.D.
	Yes	750 R	0 (8)	75 (8)	N.D.
Z1c	No	No	0 (10)	100 (5)	100 (5)
	No	750 R	0 (5)	100 (5)	N.D.
C1a	No	750R	0 (5)	100 (5)	N.D.
Z1a	Yes	No	0 (10)	80 (5)	40 (5)
None		No		89 (54)	100 (20)
		200 R		100 (8)	N.D.
		750 R		80 (10)	N.D.

to 20×10^6 cells of line D1 acquired resistance to active AA. The reactivity of line A2 to collagen type II may be explained by some cross-reactivity between antigens of *M. tuberculosis* and collagen. However, this hypothesis can only be tested with cloned A2 cells. In any case, our results indicate that lines of autoimmune effector T lymphocytes can be isolated by their response to bacterial antigens and that such cells can vaccinate against experimental arthritis. Rheumatoid arthritis, although it seems to arise spontaneously, may be triggered by an environmental agent—possibly infective (*15*)—that initiates a self-perpetuating autoimmune process.

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