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Transfer of Nonspecific Resistance to *Listeria monocytogenes* using Spleen Cells from Syphilitic Rabbits (38574)

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We have recently shown that rabbits infected with *Treponema pallidum* are resistant to challenge with *Listeria monocytogenes* (1). Suppression of the growth of *Listeria* during infection by immunologically unrelated microorganisms is usually interpreted (2–4) as an indication of acquired cellular resistance (ACR). The predominant view is that ACR is initiated by the interaction of specifically sensitized lymphocytes and homologous antigen, but nonspecifically expressed by activated macrophages. We have suggested (1) that the ability of syphilitic rabbits to suppress the growth of *Listeria* reflects stimulation of cell-mediated immunity by infection with *T. pallidum*. The purpose of the present study was to provide more direct evidence for this hypothesis by transferring spleen cells from syphilitic donors along with treponemal antigen to normal rabbits in an attempt to induce ACR to *Listeria*.

Materials and Methods. Outbred New Zealand white male rabbits weighing 2–3 kg were housed individually in rooms with an ambient temperature of 18°. Rabbits with a positive VDRL (Venereal Disease Research Laboratory) reaction were excluded because of the possibility that this resulted from a subclinical infection with *Treponema cuniculi*.

Treponema pallidum (*Nichols strain*) was obtained from the Center for Disease Control, Atlanta, GA. Rabbits regularly develop orchitis 9–14 days after intratesticular injection with 10^6–10^7 organisms. Inflamed testes were removed aseptically, minced in sterile saline and ground with a mortar and pestle. After centrifugation at 270*g* for 3 min the number of treponemes in the supernatant was determined using darkfield microscopy. All rabbits infected with *T. pallidum* for the purposes of this study received 4×10^7 organisms intravenously (IV).

Single cell suspensions of spleen cells were prepared from syphilitic and control rabbits by teasing apart spleens in minimal essential medium, filtering through nylon mesh, collecting by centrifugation at 600*g* for 10 min and washing. This procedure did not cause loss of viability as determined by the ability of cells to exclude 0.1% trypan blue. Disrupted spleen cells were prepared by slowly freezing and thawing three times.

Listeria monocytogenes that had been passed three times in rabbits was given IV in a standard inoculum of 3×10^8 organisms (1). Thirty to 32 hr later animals were asphyxiated; livers and spleens were removed aseptically and homogenized in saline. Following serial dilution aliquots were plated on brain heart infusion agar. Colonies were counted after 16–18 hr incubation at 35°. Data are reported as $\log_{10}$ of the number of *Listeria* per liver or spleen. Statistical analysis was carried out using the analysis of variance.

Results. In preliminary studies we sought to exclude the possibility that transfer of spleen cells from one rabbit to another would enhance listericidal activity by inducing a graft versus host (GVH) reaction (5). Ten normal recipients each received IV $4–6 \times 10^8$ spleen cells from ten normal donor rabbits. Forty-eight or 96 hr later five recipient rabbits and five control rabbits were challenged IV with *Listeria*: Control rabbits received no spleen cells. The rabbits were sacrificed 30–32 hr. later and the number of *Listeria* in the livers and spleens determined. Data summarized in Table I show no difference in the number of *Listeria* recovered from the tissues of recipients of allogenic lymphocytes and control rabbits. These results indicate that

transfer of spleen cells to recipients did not produce enhancement of listericidal activity within 96 hr.

We then sought to determine whether transfer of spleen cells from syphilitic to normal rabbits would confer enhanced resistance to *Listeria*. Splenic lymphocytes (4–6 × 10^8) from rabbits that had been infected IV with *T. pallidum* 30 or 80 days previously were transferred IV to recipient rabbits with (Group I) or without (Group II) 4 × 10^7 viable *T. pallidum*. Other rabbits were injected IV with 4–6 × 10^8 disrupted syphilitic spleen cells with (Group III) or without (Group IV) viable 4 × 10^7 *T. pallidum*. A fifth group received 5 × 10^8 normal spleen cells and a sixth group received 4 × 10^7 viable *T. pallidum*. Forty-eight hours later all rabbits were challenged IV with 3 × 10^8 *Listeria*. After 30–32 hr they were sacrificed and the number of *Listeria* in the livers and spleens was determined. Results of this experiment are presented in Table II. The growth of *Listeria* was suppressed in rabbits that received syphilitic spleen cells with or without *T. pallidum* ($P < 0.001$). Enhanced resistance to *Listeria* was not conferred on rabbits which received normal spleen cells or disrupted spleen cells with or without *T. pallidum*. The single exception was detection of suppression in the livers only of rabbits that received disrupted spleen cells from animals that had been infected with *T. pallidum* 30 days previously ($P < 0.05$). A partial replication of this experiment confirmed that syphilitic spleen cells transfer resistance to *Listeria* with or without simultaneous injection of viable *T. pallidum* and showed that disrupted syphilitic spleen cells did not enhance the ability of recipient rabbits to suppress the growth of *Listeria*.

TABLE I. FAILURE TO ENHANCE LISTERICIDAL ACTIVITY IN RABBITS BY TRANSFER OF SPLEEN CELLS.[a]

Tissue	Rabbits	48 hr	96 hr
Liver	Received normal spleen cells	7.108	5.901
	Control (received no spleen cells	6.562	6.390
Spleen	Received normal spleen cells	5.626	5.103
	Control (received no spleen cells)	5.299	5.185

[a] Data are expressed as mean $\log_{10}$ *Listeria* per liver or spleen. The standard error associated with each mean was 0.287 and 0.369 for the liver and spleen respectively. No significant differences were detected.

Discussion. Mackaness[6] has shown that lymphoid cells from BCG-immunized donors do not transfer nonspecific resistance to *Listeria*, even though the donors themselves are resistant. Resistant to *Listeria* is conferred on recipients when BCG is injected simultaneously with specifically sensitized lymphocytes. Our results show that transfer

TABLE II. TRANSFER OF NONSPECIFIC RESISTANCE TO *Listeria* USING SPLEEN CELLS FROM SYPHILITIC RABBITS.[a, b]

	I	II	III	IV	V	VI
Liver 30	5.301***	5.008***	6.976	5.888*	6.898	6.808
Spleen 30	3.746***	3.881***	5.730	5.027	5.589	5.256
Liver 80	4.831***	5.630**	6.222	6.190	6.278	6.757
Spleen 80	4.491***	4.805**	5.531	5.816	5.933	5.914

[a] Data are expressed as mean $\log_{10}$ *Listeria* per liver or spleen. The standard error associated with each mean was 0.310, 0.274, 0.430, and 0.249 for the liver and spleen each at 30 and 80 days respectively. Differences when compared to group V and VI are statistically significant at $P < .05$ (*), $P < .01$ (**) or $P < .001$ (***).

[b] Groups of rabbits received: I. Syphilitic spleen cells + *T. pallidum*; II. Syphilitic spleen cells; III. Disrupted syphilitic spleen cells + *T. pallidum*; IV. Disrupted syphilitic spleen cells; V. Normal spleen cells; or VI. *T. pallidum* alone. There were four animals in each group. Donors of syphilitic lymphocytes had been infected IV with 10^7 *T. pallidum* 30 or 80 days previously.

of viable spleen cells from syphilitic rabbits along with treponemes confers resistance to *Listeria* upon the recipient. That this resistance did not result from a graft-versus-host reaction (5) was shown in preliminary studies (Table I) as well as in the control groups in the experiment (Table II). Resistance was not transferred by treponemes alone nor by disrupted spleen cells either from normal or syphilitic rabbits. The ability of syphilitic spleen cells alone to transfer resistance to *Listeria* (Table II) probably results from persistence of treponemal antigen; viable *T. pallidum* can be recovered from the spleens of rabbits 30 days after IV infection and additional cell-associated treponemal antigen may also be present.

We have previously shown (1) that rabbits have enhanced ability to suppress the growth of *Listeria* three to five weeks after infection with *T. pallidum*. The experiments described in the present communication give additional evidence that an immunologic mechanism is responsible for this resistance to *Listeria*. Although we cannot exclude the possibility that bursa-dependent lymphocytes and/or macrophages contributed to the induction of ACR, we have recently shown that treating the lymphocytes with a specific anti-thymocyte serum before transfer abolishes their ability to induce ACR (R. Schell and D. Musher, unpublished observations). These data suggest that the immune response induced by *T. pallidum* is associated with activation of cell-mediated immunity.

Summary. The data presented show that spleen cells obtained from syphilitic rabbits can be used to transfer nonspecific resistance to *Listeria* to normal recipients. This supports the hypothesis that infection caused by *T. pallidum* induces a cell-mediated immune response.

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