Systemic and Local Immunological Activity of Lymph Node Cells from Mice Treated with Antilymphocyte Serum[1] (35584)

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(Introduced by Leonard J. Cole)

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Antilymphocyte serum (ALS) is an immunosuppressive agent which causes prolonged survival of allografts (1, 2) and reduces the graft-versus-host reactivity of lymphoid cells (3). The mechanism by which ALS causes these effects is not known, but it is becoming increasingly clear that immunosuppression is associated with a partial failure of ALS-exposed lymphoid cells to migrate to the sites where they are immunologically active (4, 5). We have studied a reaction in which the ALS-exposed lymphoid cells may interact locally without an apparent need for migration. This local reactivity may be produced in the skin of the X-irradiated hamster by the injection of histoincompatible lymphocyte populations (6–8). We report here that lymph node cells from mice treated with immunosuppressive ALS are significantly reactive in this assay.

Materials and Methods. Highly inbred Balb/c and C3H mice from 4–6 weeks of age and an inbred strain of Golden Syrian hamsters were employed. Six to 10-day-old (Balb/c × C3H)F1 hybrid mice were raised employing the above breeder mice. Rabbit antimouse lymphocyte serum (RAMLS) was produced in adult, New Zealand white rabbits by a sensitizing intradermal injection of 30 × 10^6 Balb/c lymph node cells in complete Freund's adjuvant followed 14 days later by an intravenous injection of 10 × 10^6 similar cells in normal saline. Rabbits were bled 7 days after the second injection. Normal rabbit sera (NRS) were also obtained. All sera used were heat-inactivated at 56° for 30 min.

The lymphocytotoxicity titers of the sera were determined by a modification of a method of Spooner and Sell (9). The immunosuppressive potency of each serum was determined by its capacity to prolong the survival of C3H skin allografts on 20 treated Balb/c recipients (10). Recipient treatment consisted in each case of injections of 0.25 ml of the appropriate serum intraperitoneally (ip) on days −1, +1, +3, and +5; skin allografting was performed on day 0. Skin allografts were considered rejected when less than 10% of the allografted skin remained soft and pliable. Statistical analysis of the data was performed by a method of Litchfield (11).

Cells for ip or intradermal (id) injection were obtained from the cervical, axillary, and inguinal lymph nodes of Balb/c and C3H donors. Donors were either untreated mice or treated mice which had been injected 3 times ip with 0.25 ml of a serum preparation 1, 2, and 4 days previously. Cell counts were performed using 0.4% trypan blue dye in Hanks' balanced salt solution (HBSS); cell suspensions for injection were prepared in HBSS.

The graft-versus-host assay of immunocompetence originally described by Simonsen

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et al. (12) was employed using the young (Balb/c × C3H)F_1 hybrid mice; these mice were injected ip with lymph node cells (20×10^6 viable lymph node cells/ml of suspension) obtained from either untreated, NRS-treated, or RAMLS-treated Balb/c donor mice. Eight days after cell injection, the hybrid mice were sacrificed, the body and spleen weights were measured, and the relative spleen weights (ratio of spleen wt:body wt) and the spleen indices (relative spleen wt of each animal divided by the mean relative spleen wt of noninjected littermate controls) were determined (13).

Local reactions caused by lymphocyte populations in the skin of the X-irradiated hamster were assessed according to a technique similar to that previously described by Ramseier and Streilein (6). Hamsters were placed in Plexiglas dosimetry cages and were subjected to 1500 R whole-body X-irradiation (irradiation factors: 250 kVp, dose rate 20 R/min in free air, distance of 1 m, ½ value layer 1.90 mm Cu). Forty-eight hr after irradiation, the hamsters were injected id with 0.1 ml of a freshly prepared lymph node cell suspension. Lymph node cells injected were either single strain populations (cells from a single donor strain consisting of either 10×10^6 Balb/c cells or 10×10^6 C3H cells in 0.1 ml of suspension) or double strain populations (cells from the two different mouse strains consisting of 5×10^6 Balb/c plus 5×10^6 C3H cells present together in 0.1 ml of suspension). Double strain populations were mixed *in vitro* prior to injection. Most of the experiments were internally controlled by the injection of each hamster with both experimental and control populations. As many as four injection sites on each hamster were utilized. Hamsters were killed 48 hr after injection; skin areas bearing the injection sites were removed; and the sizes of the reaction sites were recorded (mm of induration). The "reactivity index" of each intradermal reaction was calculated by dividing each individual reaction value by the mean reaction value caused by the injection of control double populations of normal cells.

Results. Normal rabbit serum was not lymphocytotoxic when tested against Balb/c lymphocytes; the RAMLS had a lymphocytotoxicity titer of 1:800.

TABLE I. Effect of Antilymphocyte and Normal Sera on the Graft-Versus-Host Reactivity of Lymph Node Cells.[a]

Treatment of cell donor[b]	No. of cells injected ($\times 10^6$)	No. of hosts assayed	Mean spleen index (± 1 SEM)[a]
None	1	16	1.97 ± 0.22
	2	8	3.18 ± 0.17
	5	37	4.39 ± 0.18
RAMLS[c]	5	13	1.59 ± 0.14
Normal rabbit serum	5	8	4.43 ± 0.42

[a] Assays and calculations performed as described in Materials and Methods.

[b] Cell donors injected ip three times with 0.25 ml of serum 1, 2, and 4 days prior to performing the graft-versus-host assay.

[c] Rabbit antimouse lymphocyte serum.

Median effective times (with 95% confidence limits) of skin allograft survival for the untreated, NRS-treated, and RAMLS-treated mice were 9.6 (9.1–10.2), 9.8 (9.2–10.4), and 28.5 (24.8–32.2) days, respectively.

As further evidence of the immunosuppressive potency of the RAMLS, 5×10^6 lymph node cells obtained from RAMLS-treated mice showed a significant decrease in their ability to produce splenomegaly when compared to cells obtained from either untreated or NRS-treated mice (Table I).

An analysis of the local reactivity of lymph node cells from untreated mice is presented in Table II. The reactions produced by double strain populations of those cells (mixtures of Balb/c and C3H) by definition gave a mean reactivity index (MRI) of 1.00. A reduction of the MRI was seen when either single strain populations (MRI = 0.48) or frozen-thawed, nonviable double strain populations (MRI = 0.39) were employed. These findings demonstrate that an MRI of 1.00 represents a significant local reaction dependent upon the presence of viable, histoincompatible cell populations obtained from untreated donor mice.

TABLE II. Effect of Antilymphocyte and Normal Sera on the Local Reactivity of Lymph Node Cells in the Skin of the X-Irradiated Hamster.[a]

Lymph node cell population injected	Untreated donors		RAMLS-treated donors[b]		NRS-treated donors[c]	
	No. of skin reactions	MRI (± 1 SEM)[d]	No. of skin reactions	MRI (± 1 SEM)[d]	No. of skin reactions	MRI (± 1 SEM)[d]
Single population of 10×10^6 Balb/c or 10×10^6 C3H cells (viable)	40	0.48 ± 0.06	25	0.59 ± 0.04	—	—
Double populations of 5×10^6 Balb/c and 5×10^6 C3H cells (nonviable)[e]	16	0.39 ± 0.19	11	1.17 ± 0.36	—	—
Double populations of 5×10^6 Balb/c and 5×10^6 C3H cells (viable)	20	1.00 ± 0.09	29	3.99 ± 0.37	20	1.04 ± 0.06

[a] Hamsters subjected to 1500 R (48 hr prior to injection).
[b] Rabbit antimouse lymphocyte serum-treated donors.
[c] Normal rabbit serum-treated donors.
[d] Mean reactivity index (see Materials and Methods).
[e] Rendered nonviable by freeze-thawing prior to assaying for local reactivity.

The local reactivity of lymph node cells from RAMLS-treated donors is also presented in Table II. As was true for cells from untreated donors, double strain populations of cells from RAMLS-treated donors showed significantly more reactivity (MRI = 3.99) than either the single strain populations (MRI = 0.59) or frozen-thawed, nonviable double strain populations (MRI = 1.17). Cells from RAMLS-treated donors in every case showed an increase in the MRI when compared to cells from untreated donor mice, indicating that the treatment with RAMLS also caused a nonspecific increase in local reactivity.

Discussion. These experiments demonstrate that lymph node cells obtained from mice treated with immunosuppressive RAMLS are apparently highly reactive when tested in the skin of the X-irradiated hamster. This reactivity is in part nonspecific, since killed populations of cells obtained from RAMLS-treated donors are more reactive than similar populations from untreated donors. However, double strain populations from RAMLS-treated donors showed nearly a sevenfold increase in local reactivity in comparison to single strain populations from RAMLS-treated donors (MRI increased from 0.59 to 3.99); these results show that the elevated local reactivity is dependent to a large extent upon the presence of viable, histoincompatible cell populations.

In these experiments, we have assessed the reactivity of equal numbers of cells from treated and untreated donors. However, since the lymph nodes of RAMLS-treated mice frequently contain fewer cells than the lymph nodes of untreated mice, we would expect that the reactivity of nodes from RAMLS-treated mice would be much reduced when viewed on a "per lymph node" basis (14).

There are at least three possible explanations for the increased local reactivity shown by histoincompatible cells from RAMLS-treated donors. First, the local reaction may be caused in part by the elaboration of cytotoxic substance(s), and ALS may facilitate the elaboration of these substances by causing transformation of the lymphocytes (15, 16), which then elaborate cytotoxic substance(s) (17, 18). An increase in cytotoxic substance(s) in lymph node cells obtained from RAMLS-treated mice could explain the significant local reactivity of killed cells (frozen-thawed cells) obtained from RAMLS-

treated mice. Second, ALS may act to increase the percentage of cells active in local reactions through a preferential depletion of the recirculating small lymphocytes which are involved in certain systemic immunological reactions (5, 19). Finally, ALS adversely affects the ability of lymphoid cells to migrate in the systemic circulation (4, 5). Therefore, the reactivity of those cells which do not migrate normally might be more easily detected in this local assay where there is no need for systemic migration. Indeed, the increased local reactivity might even result from the failure of lymph node cells from the RAMLS-treated mice to migrate away from the injection site; in the case of the killed cells obtained from RAMLS-treated mice, the significant reactivity noted might result from the complete lack of cell migration with the further localization of RAMLS-induced cytotoxic substance(s).

Cells obtained from RAMLS-treated mice showed a decrease in reactivity in the systemic graft-versus-host assay, but no decrease in reactivity in the local assay. This difference in systemic and local reactivity may have important implications. For example, the ALS-treated recipient of an allograft might show prolonged allograft survival, yet possess immunologically reactive cells which cannot migrate to the allograft site and/or are not reactive in allograft rejection; those cells, however, may be significantly reactive in the local assay.

Summary. An immunosuppressive antilymphocyte serum prolonged the survival of skin allografts and suppressed the systemic graft-versus-host reactivity of lymph node cells from treated mice. Those same lymph node cells, however, were highly reactive when tested in the skin of the X-irradiated hamster, and that reactivity was dependent, at least in part, upon the presence of viable, histoincompatible cell populations. We conclude that cells from the ALS-treated mice react well in that local assay while showing suppressed reactivity in the systemic assays of immunocompetence.

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