EFFECT OF RECENT ANTIGEN PRIMING ON ADOPTIVE IMMUNE RESPONSES

II. Specific Unresponsiveness of Circulating Lymphocytes from Mice Primed with Heterologous Erythrocytes*‡

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Recent studies have demonstrated that for 1 to 2 days after the injection of mice (1) or rats (2) with antigens, such as heterologous erythrocytes or histoincompatible spleen cells, there exists a specific deficiency in thoracic duct lymph of cells able to mount adoptive immune responses to these antigens. These results were interpreted in terms of antigen-induced selective recruitment of specific lymphocytes from the recirculating pool to appropriate lymphoid organs. This concept was challenged by recent findings that specific unresponsiveness after short-term priming with antigen was not restricted to thoracic duct lymphocytes (TDL)[1], but also applied to cells from spleen, mesenteric lymph nodes (MLN), and Peyer's patches (3). In the case of spleen cells, the unresponsiveness of the cells on transfer was only a temporary phenomenon. Thus, although the cells failed to respond to the priming antigen within 7 days of transfer, they gave high responses at later times. This suggested that antigen-reactive cells were present in the spleen but, for reasons not clearly apparent, they were transiently unable to express their function. The present experiments were designed to test whether recently primed TDL and MLN cells would, like spleen cells, eventually recover their capacity to respond to the priming antigen on adoptive transfer. The results showed, in fact, that the reactivity of these two cell populations did not return. This, together with the observation that intravenously injected antigen localized in

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[1] *Abbreviations used in this paper:* ASRL, antigen-specific recruitment of lymphocytes; HRC, horse erythrocytes; MLN, mesenteric lymph nodes; PFC, plaque-forming cells; 19S PFC, direct plaque-forming cells; 7S PFC, indirect (developed) plaque-forming cells; SRC, sheep erythrocytes; TDL, thoracic duct lymphocytes; TxBM, thymectomized, irradiated, marrow protected.

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the spleen but not in the MLN, suggested that antigen, when administered intravenously and at an appropriate dose, did indeed lead to selective recruitment of lymphocytes from the recirculating pool to the spleen.

Materials and Methods

Mice.—Highly inbred CBA/J mice were used. Their origin and maintenance has been described elsewhere (3).

Cell Suspensions.—TDL were collected by the method of Sprent (4). Suspensions of spleen and MLN cells were prepared as described previously (3). Erythrocytes were obtained from the jugular veins of sheep (SRC) and horses (HRC) and stored in Alsever's solution. When required they were washed three times in saline and resuspended to an appropriate volume. In general, 10^9 erythrocytes were contained in 0.2 ml of a 25% suspension and 5×10^8 cells in 0.1 ml of a 25% suspension. The number of erythrocytes injected varied in different experiments and is shown in the tables.

Injections.—All suspensions of lymphoid cells and heterologous erythrocytes were injected intravenously via the lateral tail vein.

Thymectomy.—Mice were thymectomized at 6 wk of age according to the technique outlined by Miller (5). Thymectomized mice were irradiated 3 wk after the operation.

Irradiation.—Mice were exposed to 750-R total body irradiation as described previously (3). All irradiated mice received polymyxin B (100,000 IU/liter) and neomycin (10 mg/liter) in the drinking water.

Labeling of Erythrocytes with ^{51}Cr.—SRC were washed three times and then incubated for 30 min at 37°C with ^{51}Cr (obtained as $Na_2{}^{51}CrO_4$, specific activity = 338 mCi/mg of Cr, Radiochemical Center, Amersham, Buckinghamshire, England) at a concentration of 30 μCi/ml of a 25% suspension of the cells in normal saline. After incubation, the cells were washed four times and transferred intravenously in 0.25 ml to normal mice. Various organs were removed from the mice at intervals. Levels of radioactivity in the organs were measured as described elsewhere (4) and expressed as a percentage of the counts present in an aliquot of the injected cells.

Plaque-Forming Cell (PFC) assays.—Numbers of 19S (direct) and 7S (indirect) PFC to SRC and HRC in spleens were measured as described previously (3).

RESULTS

Specific Unresponsivenss of TDL Transferred from Normal Mice Injected 1 day Before with Heterologous Erythrocytes.—In previous studies, the adoptive response of TDL to heterologous erythrocytes was investigated in thymectomized, irradiated marrow-protected (TxBM) mice (1). When TDL were collected from donors injected 1 day before with 5×10^8 SRC given intravenously, the adoptive response of the cells to SRC was specifically reduced, but not abolished. It has now been found possible to abolish this response completely: (*a*) by giving a higher dose of antigen to prime the TDL donors and (*b*) by using irradiated rather than TxBM mice as the cell recipients.

Normal mice were injected intravenously with 10^9 SRC, 10^9 HRC, or saline. Thoracic duct fistulae were produced in these mice 20 h later and TDL collected over a 12-h period, i.e., from 22 to 34 h after injection of antigen. TDL, pooled separately from each of the three groups, were injected intravenously at a dose of 1.5×10^7 cells together with 5×10^8 SRC and 5×10^8 HRC into

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irradiated (750 R) mice. The numbers of PFC to SRC and HRC detected in the spleens of the recipients 7 days after cell transfer are shown in Table I. High numbers of 19S and 7S PFC to both SRC and HRC were found in recipients of TDL from unprimed mice. TDL from mice "primed" to SRC 1 day before, however, failed to respond to SRC on transfer, although they responded well to HRC. Similarly, TDL from mice primed to HRC produced high numbers of PFC to SRC, but not to HRC.

The above experiment showed clearly that the adoptive response of TDL to a particular antigen could be specifically abolished by priming the TDL donors with this antigen 1 day before the cells were transferred. The protocol used for this experiment was thus adopted for all subsequent experiments unless otherwise indicated. It should be mentioned that small numbers of 19S and 7S PFC to both SRC and HRC were often detected in irradiated mice given antigen but not lymphocytes. The geometric mean of these values (which usually did not exceed 100 PFC/spleen) were subtracted from the data shown in Table I and from all subsequent experiments.

The effect of varying the number of TDL adoptively transferred from mice primed with antigen for 1 day is shown in Fig. 1. It is evident that the unresponsiveness of TDL to the priming antigen could not be overcome by increasing the numbers transferred from 5×10^6 to 3×10^7; only the 7S responses are shown; the 19S responses gave similar results.

The dose of antigen required to induce the phenomenon shown in Table I

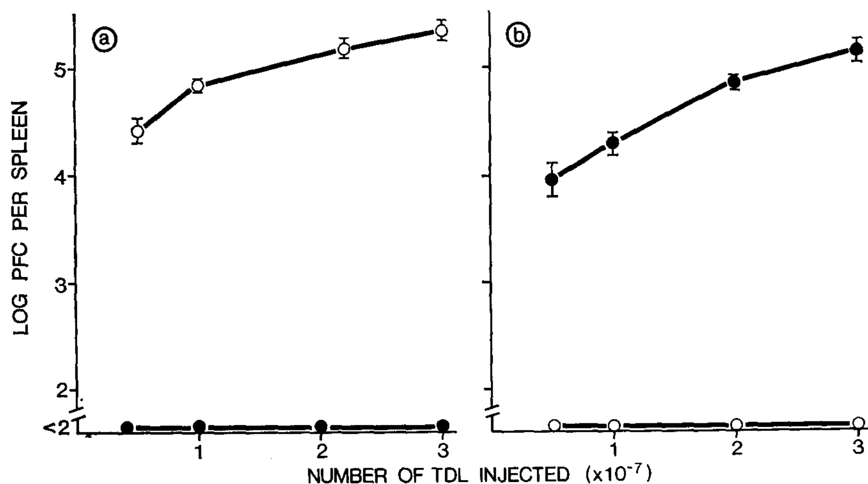

FIG. 1. Numbers of 7S PFC to SRC (group *a*) and HRC (group *b*) in spleens of irradiated mice 7 days after intravenous injection of 5×10^8 SRC and 5×10^8 HRC given together with varying numbers of TDL from mice injected 1 day previously with 10^9 SRC (●) or 10^9 HRC (○). Geometric mean of the results obtained from 4 to 6 mice are given and vertical bars represent upper and lower limits of SE.

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is illustrated in Fig. 2. Priming with small doses of SRC (10^6 or 10^7 erythrocytes) did not reduce the adoptive response to SRC of TDL taken from these mice 1 day later. A dose of 10^8 SRC, however, caused a partial reduction in the

TABLE I

Numbers of PFC in Spleens of Irradiated Mice Injected with TDL from Mice Primed 1 day before with SRC or HRC**

Cells transferred together with SRC‡ and HRC‡	PFC per spleen at 7 days			
	Anti-SRC		Anti-HRC	
	19S	7S	19S	7S
1.5 × 10^7 unprimed TDL	6,640§ (8,970–4,920)	91,390 (100,530–83,080)	3,640 (4,910–2,700)	73,530 (83,090–65,070)
1.5 × 10^7 SRC-primed TDL	30 (50–10)	0	2,790 (3,240–2,400)	67,180 (79,950–56,460)
1.5 × 10^7 HRC-primed TDL	5,280 (6,130–4,550)	67,180 (79,950–56,460)	70 (120–40)	7 (20–2)

* Donors of TDL were primed with 10^9 erythrocytes (0.2 ml of 25% solution) intravenously; TDL were collected between 22 and 34 h after antigen priming.

‡ 5 × 10^8 erythrocytes (0.1 ml of 25% solution) given intravenously.

§ Geometric mean, upper and lower limits of SE; 6 mice per group.

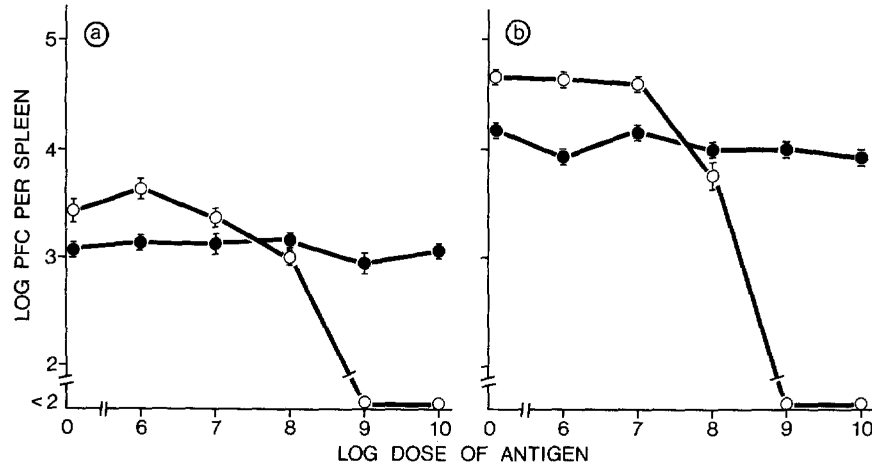

FIG. 2. Numbers of 19S (group *a*) and 7S (group *b*) PFC in spleens of irradiated mice 7 days after intravenous injection of 5 × 10^8 SRC and 5 × 10^8 HRC given together with 10^7 TDL from mice primed 1 day previously with varying numbers of SRC injected intravenously. ○ = anti-SRC PFC; ● = anti-HRC PFC. Geometric means of the results obtained from 4 to 6 mice are given and vertical bars represent upper and lower limits of SE.

response to SRC. With 10^9 and 10^{10} SRC, both the 19S and 7S responses to SRC were abolished. The response of TDL to HRC was not altered by any dose of SRC given to the cell donors before transfer.

Specific unresponsiveness after adoptive transfer was also demonstrable

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with TDL from mice primed to antigen several weeks previously, provided that an additional injection of the priming antigen was given to the cell donors 1 day before transfer. The results of such an experiment are shown in Table II. Groups of normal mice were injected intravenously with 5×10^8 SRC or 5×10^8 HRC. 6 wk later, some of the mice in each group were given an additional injection of 10^9 erythrocytes of the priming antigen; other mice received a higher dose of antigen, i.e., 10^{10} erythrocytes; the remainder were not given additional antigen. One day later, 10^7 TDL from mice of each of the 6 groups were trans-

TABLE II

Numbers of PFC in Spleens of Irradiated Mice Injected with TDL from Donors Given SRC or HRC 6 wk Previously: Effect of Injecting Donors with a Second Dose of Antigen 1 day before Adoptive Transfer*

Antigen given to TDL donors before adoptive transfer		Antigen injected‡ together with transferred TDL	PFC per spleen at 7 days			
			Anti-SRC		Anti-HRC	
6 wk before transfer‡	1 day before transfer§		19S	7S	19S	7S
SRC	none	SRC + HRC	30,340‖ (39,750–29,390)	438,330 (547,910–350,660)	2,910 (3,750–2,260)	35,820 (44,750–28,660)
SRC	SRC(10^9)	SRC + HRC	30 (40–20)	3,740 (5,310–3,170)	2,490 (3,530–1,750)	27,230 (31,310–19,880)
SRC	SRC(10^{10})	SRC + HRC	0	0	2,530 (2,990–2,140)	24,180 (31,940–21,460)
HRC	None	SRC + HRC	5,230 (5,750–4,760)	65,570 (75,140–57,020)	11,160 (12,150–10,240)	195,980 (215,580–178,160)
HRC	HRC(10^9)	SRC + HRC	10,030 (10,080–9,370)	70,470 (92,320–53,790)	370 (410–340)	4,760 (6,290–3,610)
HRC	HRC(10^{10})	SRC + HRC	15,120 (22,220–10,290)	206,760 (254,320–168,100)	190 (250–150)	0

* 10^7 cells injected intravenously.

‡ 0.1 ml of 25% suspension of erythrocytes (5×10^8) given intravenously.

§ 0.2 ml of 25% suspension of erythrocytes (10^9) or 1.0 ml of 50% suspension of erythrocytes (10^{10}) given intravenously.

‖ Geometric mean, upper and lower limits of SE, 6 mice per group.

ferred to irradiated recipients together with both SRC and HRC and numbers of PFC were measured at 7 days. When a dose of 10^9 erythrocytes was given to the TDL donors 1 day before transfer, the adoptive response to the priming antigen was specifically reduced, but not abolished; with 10^{10} erythrocytes, however, complete suppression was observed (Table II).

Comparison of the Unresponsive State Induced in TDL, Spleen, and MLN by Short-Term Priming with Antigen.—The above findings confirmed earlier work that TDL, like spleen, MLN, and Peyer's patch cells, specifically failed to respond to a particular antigen on adoptive transfer if a high dose of this antigen had been administered to the cell donors 1 day before (3). In the case of spleen cells, unresponsiveness was only temporary, i.e., the transferred cells

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eventually regained their capacity to respond to the priming antigen. It therefore became important to determine whether this return of responsiveness shown by spleen cells also applied to TDL.

Spleen cells from mice primed with antigen 1 day before had been found to regain their capacity to respond to the priming antigen when PFC in the cell recipients were assayed at 11 days instead of at 7 days (3). The effect of varying the time of the PFC assay in recipients of 1-day-primed TDL is shown in Fig. 3. TDL from mice primed 1 day before with SRC, while responding well

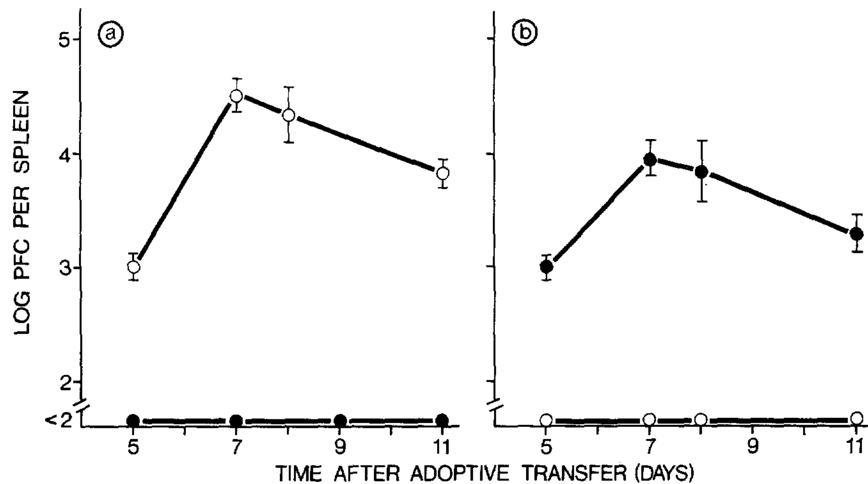

FIG. 3. Numbers of 7S PFC to SRC (group *a*) and HRC (group *b*) in spleens of irradiated mice at various times after intravenous injection of 5×10^8 SRC and 5×10^8 HRC given together with 10^7 TDL from mice injected 1 day previously with 10^9 SRC (●) or 10^9 HRC (○). Geometric means of the results obtained from 4 to 6 mice are given and vertical bars represent upper and lower limits of SE.

to HRC on transfer, failed to give detectable 7S (Fig. 3) or 19S (data not shown) responses to SRC when PFC were assayed after 5, 7, 8, or 11 days. Similarly, TDL from mice primed to HRC responded well to SRC on transfer but did not respond to HRC at any stage.

1-day-primed spleen cells had been found to respond normally to the priming antigen if antigen challenge of the transferred cells was delayed for 5 or more days and PFC were measured 7 days later (3). To determine whether TDL would respond under such circumstances, the following experiment was performed: TDL from mice primed 1 day before with SRC or HRC were transferred to irradiated mice. These mice were then injected with both SRC and HRC, either (*a*) at the time of cell transfer, (*b*) at 7 days, or (*c*) at 7 and 14 days. PFC were then measured 7 days after antigen challenge or rechallenge, i.e., at 7, 14, or 21 days. Since TDL lack hemopoietic stem cells, a small dose

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(10^6 cells) of bone marrow was given to the cell recipients at 4 days. To prevent *de novo* formation of T cells from stem cells, all recipients were thymectomized before irradiation.

The results of the above experiment, performed in parallel with a comparable experiment using spleen cells instead of TDL, are shown in Table III. Whereas, in the case of 1-day-primed spleen cells, a delay in antigen challenge of the transferred cells led to a complete recovery of the response to the priming antigen, no such recovery was observed in recipients of TDL, even with challenge at 7 and 14 days and PFC assay at 21 days. There appeared to have been some differentiation of stem cells into B cells by 21 days. Thus, in control groups of mice given normal thymus cells instead of TDL, appreciable PFC responses were observed at 21 days, although not at 14 days. The lack of a response to the priming antigen in recipients of TDL at this time must therefore have resulted from a lesion in at least the T-cell lineage.

An experiment similar in design to that shown in Table III was also performed with MLN cells. The results showed that MLN cells from 1-day-primed mice failed to respond to the priming antigen within 7 days, either when antigen challenge was given at the time of cell transfer or when challenge was delayed for 7 days (Table IV).

The above experiments indicated, therefore, that whereas the unresponsiveness of spleen cells on transfer was only of short duration, that of TDL and MLN cells was not. Could this be related to the localization of the priming injection of antigen? The distribution of radioactivity in normal mice, injected intravenously with 10^9 ^{51}Cr-labeled SRC, is shown in Table V. It is clear that whereas approximately 11% of the counts contained in the injected cells were trapped in the spleen, no detectable counts were found in the MLN at 4, 24, or 48 h postinjection.

DISCUSSION

TDL from mice primed with heterologous erythrocytes 1 day previously were found to be totally and specifically unresponsive to the priming antigen after adoptive transfer to irradiated mice (Table I). These cells, unlike spleen cells, failed to regain their reactivity when they were left for 1–2 wk after transfer before challenge with antigen (Table III). Complete ablation of the adoptive response occurred only when the TDL donors were primed with a high dose of antigen (10^9 erythrocytes or greater) (Fig. 2). This might explain why, in earlier studies of ours (1) and others (2), where relatively lower priming doses of antigen were used in systems similar to the present, only partial states of unresponsiveness were observed. Ford and Atkins (6) reported that an almost complete depletion of specifically reactive lymphocytes resulted when parental TDL were passaged for 1–2 days through sublethally irradiated F_1 rats. The high dose of antigen provided by the irradiated hosts in their system was presumably an important factor in the degree of depletion observed.

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TABLE III

Numbers of PFC in Spleens of Irradiated Mice Injected with TDL or Spleen Cells from Donors Primed with SRC or HRC* 1 day Previously: Effect of Delay in Antigen Challenge of the Transferred Cells*

Cells transferred‡	7S§ PFC per spleen					
	At day 7 (SRC + HRC at day 0)		At day 14 (SRC + HRC at day 7)		At day 21 (SRC + HRC at day 7, day 14)	
	Anti-SRC	Anti-HRC	Anti-SRC	Anti-HRC	Anti-SRC	Anti-HRC
Unprimed spleen	98,060‖ (106,890–89,970)	36,830 (42,360–32,030)	53,300 (71,950–39,480)	20,750 (23,860–18,040)	—	—
SRC-primed spleen	110 (400–30)	113,660 (138,670–93,160)	70,450 (73,980–67,100)	15,400 (17,250–13,750)	—	—
HRC-primed spleen	67,180 (82,630–54,620)	20 (60–5)	65,090 (83,970–50,460)	36,460 (37,560–35,400)	—	—
Unprimed TDL	65,760 (86,800–49,820)	21,560 (25,020–18,590)	42,340 (50,390–35,580)	11,970 (15,920–9,000)	25,910 (29,280–22,930)	22,690 (31,090–16,570)
SRC-primed TDL	0	32,970 (36,600–29,700)	0	28,920 (35,000–23,900)	0	39,690 (52,080–30, 350)
HRC-primed TDL	102,680 (110,350–94,610)	0	43,920 (68,950–27,980)	0	34,790 (58,100–20,830)	0
Normal thymus	—	—	0	0	4,860 (5,930–3,980)	2,210 (2,280–2,150)

* 0.2 ml of 25% suspension of erythrocytes (10^9) given intravenously.

‡ 10^7 TDL or 3×10^7 viable spleen cells were transferred intravenously; recipients of TDL were given, 4 days after cell transfer, an intravenous injection of 10^6 syngeneic bone marrow cells treated in vitro with anti-θ serum and complement (3); antigen challenge of the transferred cells at the times indicated consisted of an intravenous injection of 5×10^8 SRC and 5×10^8 HRC; all cell recipients were thymectomized 3 wk before irradiation. Thymus cells were transferred at a dose of 10^8 viable cells; recipients of thymus cells received 10^6 bone marrow cells 4 days later.

§ 19S PFC values gave similar results.

‖ Geometric mean, upper and lower limits of SE, 4–6 mice per group.

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The secondary response of TDL from mice primed with antigen 6 wk before could be specifically abolished by injecting the cell donors with an additional dose of the same antigen 1 day before transfer (Table II). In order to induce total unresponsiveness in this situation, the second dose of antigen had to be very high, i.e., 10^{10} erythrocytes; this was 10-fold higher than the dose re-

TABLE IV

Numbers of PFC in Spleens of Irradiated Mice Injected with MLN Cells from Donors Primed with SRC or HRC* 1 day Previously: Effect of Delay in Antigen Challenge of the Transferred Cells*

Cells transferred‡	7S‖ PFC per spleen			
	At day 7 (SRC + HRC at day 0)		At day 14 (SRC + HRC at day 7)	
	Anti-SRC	Anti-HRC	Anti-SRC	Anti-HRC
SRC-primed MLN	0	11,930 (14,080–10,110)	0	22,490 (24,740–20,450)
HRC-primed MLN	19,100§ (23,300–15,660)	0	37,950 (50,480–28,540)	20 (80–8)

* 0.2 ml of 25% suspension of erythrocytes (10^9) given intravenously.

‡ 2×10^7 viable cells transferred intravenously; cell recipients were thymectomized before irradiation, protected with marrow cells at day 4 and challenged with antigen as described for recipients of TDL in legend to Table III.

§ Geometric mean, upper and lower limit of SE, 5 mice per group.

‖ 19S PFC values gave similar results.

TABLE V

*Distribution of Radioactivity in Normal CBA Mice Injected Intravenously with ^{51}Cr-labeled SRC**

Time after injection of labeled SRC	% of injected radioactive counts‡ in:						Total % radioactivity recovered
	0.5 ml blood	Lung	Liver	Spleen	MLN	Intestines	
h							
4	0.6§	0.9	19.0	11.0	<0.1	1.1	32.6
24	0.1	0.3	20.5	10.6	<0.1	0.6	32.1
48	0.2	0.3	13.3	11.0	<0.1	0.7	25.6

* 0.2 ml of 25% suspension of erythrocytes (10^9).

‡ Calculated by reference to an aliquot of the injected cells.

§ Arithmetic mean of data obtained from four mice; the SE were within 5–15% of the mean.

quired to produce unresponsiveness in mice not previously primed with antigen. In this respect it is of interest that immunological memory in T cells (although not in B cells) may simply represent a quantitative increase in antigen-reactive cells (7). Thus, the dose of antigen required to induce unresponsiveness in TDL 1 day before transfer may be directly related to the absolute numbers of responsive cells present in the recirculating lymphocyte pool.

The observation that the unresponsiveness induced in TDL by short-term

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antigen priming was long-lasting while that in spleen was only temporary is of some importance. Thus, it strongly suggests that different mechanisms governed the unresponsiveness in the two populations. The data are conveniently explained if, as mentioned in the introduction, injection of antigen does indeed lead to a specific sequestration of recirculating lymphocytes by the spleen. According to this concept, which can be termed "antigen-specific recruitment of lymphocytes" (ASRL) (cf. reference 8), TDL collected at such times should be permanently unresponsive to the antigen in question as a result of a deficiency of specifically reactive lymphocytes. Spleen cells, by contrast, should be enriched in cells with such specificity and therefore mount high responses to the antigen on transfer. This in fact was observed, with the proviso, however, that, in the case of spleen, the cells were allowed to remain in their irradiated hosts for 1 wk before being challenged with antigen. The reason why the spleen cells were initially unresponsive on transfer is not clear. It is probable that the antigen-reactive cells, though presumably present in the spleen because of ASRL, were in a transformed state as a result of antigen activation. As postulated elsewhere, this activated state of the cells, together with undefined factors associated with the adoptive transfer procedure per se, might temporarily prevent restimulation of the cells (3).

Antigen localization studies with ^{51}Cr-labeled SRC indicated that with a dose of 10^9 labeled cells about 10% of the injected antigen became trapped in the spleen; virtually none, however, could be detected in the MLN (Table V). Thus, according to the concept of ASRL, the failure of MLN cells from recently primed mice to respond to the priming antigen on transfer would reflect a deficiency of antigen-reactive cells, these cells having been mobilized in the previous 24 h to the antigen-rich environment of the spleen. If this were correct, then MLN, like TDL, should fail to recover their reactivity to the priming antigen on transfer. This was indeed observed, at least over the 2-wk period studied (Table IV).

Several studies consistent with the concept of ASRL have already been mentioned (1, 2, 6). This concept has also been invoked to explain recent experiments of Ford (9) and Hay, Cahill, and Trnka (10). Ford perfused isolated rat spleens with TDL from syngeneic rats immune to tetanus toxoid. These cells, when harvested after perfusion for 10 h, produced high responses to the priming antigen on adoptive transfer. However, when tetanus toxoid was injected into the spleens during the perfusion, the adoptive response of the cells to this antigen was substantially and specifically reduced. Hay et al. cannulated efferent lymphatics of popliteal lymph nodes of sheep and, by one-way mixed lymphocyte culture, studied the effect of infusing allogeneic lymphocytes into the afferent lymphatics. Within the 1st day of infusion, the host lymphocytes responded well against cells of donor origin. Shortly afterwards, however, the host cells became totally and specifically unresponsive to the donor antigens; this applied not only to cells collected from the infused node but also to those obtained from the contralateral node. Furthermore, recent experiments have shown

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that the systemic unresponsive state of the host can be prolonged indefinitely by removing the injected node after 1–2 days (Cahill, personal communication). If, as the authors suggest, the node removed at this time contains, as a result of ASRL, the host's entire complement of specifically reactive lymphocytes, the clinical implications of their data are obvious.

It is important to distinguish ASRL from the non-specific alteration in the rate of cell migration through lymph nodes which may follow local injection of antigen (11). This phenomenon, which may also occur in the spleen (9), is probably a sequel to inflammation induced by the antigen (J. Hay and R. Cahill, personal communication).

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SUMMARY

When thoracic duct lymphocytes (TDL) or mesenteric lymph node (MLN) cells from mice primed 1 day before with either sheep erythrocytes (SRC) or horse erythrocytes (HRC) were transferred together with both SRC and HRC to irradiated mice, antibody responses measured 7 days later could not be detected to the priming antigen but were high to the other antigen. Furthermore, this unresponsiveness of TDL and MLN to the priming antigen could not be abrogated by delaying antigen challenge of the transferred cells for 1–2 wk.

Previous work had shown that short-term priming with antigen also induced specific unresponsiveness in spleen cells on adoptive transfer. Unresponsiveness in these cells, however, was only of temporary duration, full recovery in the reactivity of the cells being observed when challenge with the priming antigen on transfer was delayed for 5 or more days. Since the present work showed that such recovery from initial unresponsiveness on transfer was unique to spleen cells and did not apply to TDL or MLN, it appeared that different mechanisms were responsible for the unresponsiveness in the three populations. It is proposed that the unresponsiveness detected in TDL and MLN cells in the present study resulted from a deficiency of antigen-reactive cells, these cells having been recruited to the spleen, i.e., a region of antigen concentration. This concept of antigen-induced selective recruitment of circulating lymphocytes was supported by evidence that ^{51}Cr-labeled heterologous erythrocytes indeed localized largely in the spleen after intravenous injection but not in MLN.

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