REGULATION OF THE T-CELL RESPONSE TO ECTROMELIA VIRUS INFECTION

I. Feedback Suppression by Effector T Cells

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Recovery of mice from primary infection with ectromelia virus, a natural mouse pathogen, has been shown to depend on cell-mediated immunity (CMI)[1] (1–3). The specific effector cells are known to be thymus-derived lymphocytes (T cells) (2–6). CMI can be measured in two ways. First, by transferring immune T cells into preinfected recipients and quantitating virus clearance in vivo (2, 3, 6). Second, by measuring cytolysis of virus-infected target cells caused by cytotoxic T cells in vitro (4, 5). These two different functions may be mediated by the same T-cell subset since they both require compatibility at the *K* or *D* region of the *H-2* gene complex between T-cell donors and recipients[2] or target cells (7), respectively, and display similar kinetics of effector cell production in the spleen.[3] The primary response, as measured by the generation of cytotoxic T cells in the spleen, is detectable 2 days after infection, reaches a peak on days 5–6, and then declines to low levels by day 10 (4).

Furthermore, when immune spleen cells taken from mice infected 12 or more days previously are cultured with virus-infected spleen cells in vitro, a strong secondary response is elicited as indicated by the generation of potent cytotoxic T cells.[3] This response, however, is not observed with 8 or 10-day immune cells.[3] This suggests that the mechanisms responsible for the decline of the primary response (after day 6) and for the period where the cells are 'refractory' to secondary stimulation (days 8–10 postimmunization) are no longer exerting their effects after day 12 when a strong secondary response can be produced in vitro. The importance of a secondary T-cell response in resisting reinfection is not known, but it is potentially significant if virus evades neutralization by antibody and succeeds in spreading from the portal of entry to crucial target organs such as liver and spleen (1–3). Furthermore, little is known about the regulation of cell-mediated immune responses in general, and it is thus of considerable interest to determine what factors may be involved in controlling the primary and secondary CMI response to a natural virus infection.

This report describes the results of investigations of mechanisms involved in regulating the primary immune response to ectromelia virus infection. The rationale of our approach is as follows: if there are regulatory factor(s) present in the spleen and/or serum of infected animals, their effects can be studied by

[1] *Abbreviations used in this paper:* CMI, cell-mediated immunity; F-15, Eagle's minimum essential medium with nonessential amino acids.

[2] Kees, U., and R. V. Blanden. 1976. A single genetic element in *H-2K* affects T-cell antiviral function in poxvirus infection. *J. Exp. Med.* In press.

[3] Gardner, I., and R. V. Blanden. Manuscript in preparation.

injecting the spleen cells or serum into recipient animals after an immunizing dose of infectious virus. It was found that ectromelia-immune spleen cells and serum when injected into preinfected mice suppressed the primary response as indicated by decreased cytotoxic cell activity in the spleen. Further analysis suggested that such a suppressive effect is most probably due to removal of virus and infected cells bearing the antigens necessary to induce the response.

Materials and Methods

Animals. Mice of various strains were obtained from breeding colonies in this laboratory. They were used at 7–10 wk of age. Unless stated otherwise, CBA/H mice were used.

Virus. Virulent Moscow strain and attenuated Hampstead Egg strain ectromelia virus were used. The method of growing and titrating these strains has been described previously (4).

Bacteria. The preparation of *Listeria monocytogenes* inocula has been described previously (2).

Immunization of Mice. Mice were infected intravenously (i.v.) with 2×10^4 PFU (plaque forming units) of Hampstead Egg strain ectromelia virus (unless otherwise stated) or with 10^3 viable *Listeria monocytogenes.* Spleens were removed at various times after immunization, and groups of three organs were pooled for the preparation of cell suspensions.

Spleen Cell Suspensions. Spleens were treated as described previously (4, 8), and cells were finally suspended in Eagle's minimum essential medium with nonessential amino acids (F-15).

Anti-θ Serum. Anti-θ serum was obtained from AKR/J mice injected intravenously with 2.5×10^7 thymocytes from C57BL/6 mice. Sera were collected 1 wk later and tested for specificity against CBA/H T cells as described previously (8). Immune spleen cells were suspended at 5×10^7 viable cells/ml in a one-tenth dilution of anti-θ serum and incubated at room temperature for 30 min. After washing twice, the cells were resuspended in a one-fourth dilution of absorbed rabbit complement in F-15 and again incubated at 37°C for 30 min (complement was absorbed with outbred and CBA/H mouse spleen cells until all nonspecific activity was removed). After two more washes, the cells were resuspended in F-15 and viability determined by trypan blue exclusion.

Cell Culture. Continuous line mouse fibroblasts (L-929) and mastocytoma cells (P-815-X2) were grown as described previously (4).

Cytotoxic Assay. The method used was essentially similar to that described previously (4) except that L-929 target cells were labeled with ^{51}Cr, infected with virus, and dispensed while in suspension (as for P-815-X2 cells).

Thymectomy. Mice were thymectomized at 8 wk of age by the method of Miller (9).

Results

Suppressive Activity of Ectromelia-Immune Spleen Cells and Serum. Ectromelia-immune spleen cells and serum obtained 7 or 10 days after immunization of donors were injected into recipient mice 1–2 h after an immunizing dose of virus. Spleen cells of recipients were then assayed 6 days later (the time of the peak of the primary response) (4) for cytotoxic activity against virus-infected target cells. Ectromelia-immune spleen cells, and to a lesser extent serum, had a suppressive effect on the primary response as indicated by decreased cytotoxic cell activity in the spleens of recipients (Table I). 7- or 10-day immune cells did not seem to differ markedly in their ability to suppress, but 10-day immune serum appeared to be superior to 7-day serum; normal cells and serum had no suppressive effect (Table I).

Suppressive Effects of Immune Cells and Serum Injected at Different Times Relative to Immunization. The suppressive effect described above was observed when the cells or serum were injected 1–2 h after an immunizing dose of virus. When the injection was delayed further, the suppressive effect was observed when they were injected 1 day, but not 2 days, after virus (Table II).

TABLE I
Suppression of Primary Response by 7- and 10-Day Immune Spleen Cells and Serum

Serum or cells injected*	Percent specific ^{51}Cr release from infected L-929 cells‡	Percent suppression§
None	57.8‖ ± 2.2	0
Normal serum	54.7 ± 1.3	5.4
Normal cells	56.6 ± 1.1	2.1
Immune serum (7-day)	52.4 ± 3.6	9.3
Immune cells (7-day)	5.8 ± 1.2	90.0
None	44.0 ± 2.7	0
Normal serum	46.7 ± 1.4	0
Normal cells	50.2 ± 4.3	0
Immune serum (10 day)	23.4 ± 4.0	46.8
Immune cells (10 day)	7.8 ± 1.3	82.3

* CBA/H mice were injected with 5×10^4 PFU attenuated ectromelia virus; 1–2 h later they were injected with normal or immune serum (0.6 ml per mouse i.v.), normal or immune spleen cells (1.2×10^8 cells per mouse i.v.), or received no further treatment. Spleen cells from pools of three mice were then assayed 6 days later for cytotoxic activity as described in the text. Donors of cells and serum examined for suppressive activity were immunized as described in the text.

‡ Means ± SE of the mean in groups of four wells with spontaneous release (release in wells containing only medium and target cells) subtracted. Killer to target ratio was 100:1.

§ Percent specific ^{51}Cr release from those spleen cells obtained from mice receiving virus only is taken as 100% release (or 0% suppression).

‖ Significantly greater release than from uninfected L-929 cells ($P < 0.001$).

Ability of Different Immune Spleen Cell Populations to Suppress the Primary Response. The kinetics of the production of cells with suppressive effect were investigated. Ectromelia-immune spleen cells were obtained at various times after primary immunization and their suppressive activity assayed. Cells were again injected into recipients 1–2 h after virus, and cytotoxic activity in the spleen was determined 6 days later. Suppressive activity was detected with 4-day immune cells, reached a maximum with 6-day immune cells, and then declined (Fig. 1).

Specificity of Suppressive Effect. The ability of immune spleen cells and serum to suppress appears to be specific; spleen cells and serum from mice infected with *Listeria monocytogenes*, a bacterium that induces a strong T-cell response (8), had little suppressive activity (Table III). It was also found that allogenic immune spleen cells (from BALB/c mice) did not possess suppressive activity in CBA/H recipients, and that F_1 hybrid cells (CBA/H × BALB/c) appeared to be less effective than parental cells (Table IV). BALB/c immune cells had potent suppressive activity when injected into BALB/c (syngeneic) recipients, however, and F_1 cells once again appeared to be less effective (Table IV). Immune spleen cells from A.TL mice (which share *I*-region genes of the *H-2* gene complex, but not *K*- or *D*-region genes with CBA/H) had little suppressive activity in CBA/H mice, but were potent suppressors of the primary response when injected into BALB/c mice (which share *D*-region genes with A.TL) (Table IV). This result suggests that *I*-region homology in the *H-2* gene complex is

TABLE II

Suppressive Effect of 10-Day Immune Cells and Serum Injected at Different Times Relative to Immunization

Serum or cells injected*	Time of injection after virus	Percent specific ^{51}Cr release from infected L-929 cells‡	Percent suppression§
None	—	44.0 ‖ ± 2.7	0
Normal serum	2 h	46.7 ± 1.4	0
Normal cells	2 h	50.2 ± 4.3	0
Immune serum	2 h	23.4 ± 4.0	46.8
Immune cells	2 h	7.8 ± 1.3	82.3
None	—	31.3 ‖ ± 3.0	0
Normal serum	1 day	29.3 ± 1.2	6.4
Normal cells	1 day	38.5 ± 1.1	0
Immune serum	1 day	15.4 ± 2.0	50.8
Immune cells	1 day	20.3 ± 1.3	35.1
None	—	48.1 ‖ ± 3.7	0
Normal serum	2 days	50.4 ± 1.6	0
Normal cells	2 days	54.7 ± 4.0	0
Immune serum	2 days	60.6 ± 3.3	0
Immune cells	2 days	54.8 ± 1.9	0

* Experimental procedure as for Table I except that cells or serum were injected into recipient mice at various times (2 h, 1 day, or 2 days) after injection of 5×10^4 PFU attenuated ectromalia virus.

‡, §, ‖ As for Table I.

neither necessary nor sufficient for suppression to occur, but *D*-region homology is sufficient.

Effect of Anti-θ Treatment on Suppressive Activity. To determine the nature of the cells involved in suppression, 6-day immune spleen cells were treated with anti-θ serum before injection into recipient mice 1–2 h after virus. Anti-θ treatment eliminated the ability of 6-day immune cells to suppress the response (Table V). This result suggests that the suppressive cells are T cells or that the mechanism of suppression is T-cell dependent.

Dose Response. The effect of varying both the dose of suppressive spleen cells and the dose of immunizing virus used in cell recipients was investigated. Suppression was observed with 2×10^7 immune cells per mouse and was not improved with higher cells doses (Table VI). Varying the virus dose had no effect on suppression within the dose range tested (Table VI).

Kinetics of Cytotoxic Activity in the Spleen after Injection of Suppressive Cells. In previous experiments, cytotoxic activity in the spleens of recipient mice was assayed 6 days after injection of suppressive cells. To determine the kinetics of cytotoxic activity in the spleens of recipient mice, they were assayed at various times after injection of suppressive cells and virus. Significant cytotoxic activity was observed 2–4 days after injection, declined significantly on day 5, and increased again on day 6 (Fig. 2). The cytotoxic activity detected on days 2–4 probably represents the continued proliferation of the cytotoxic cells

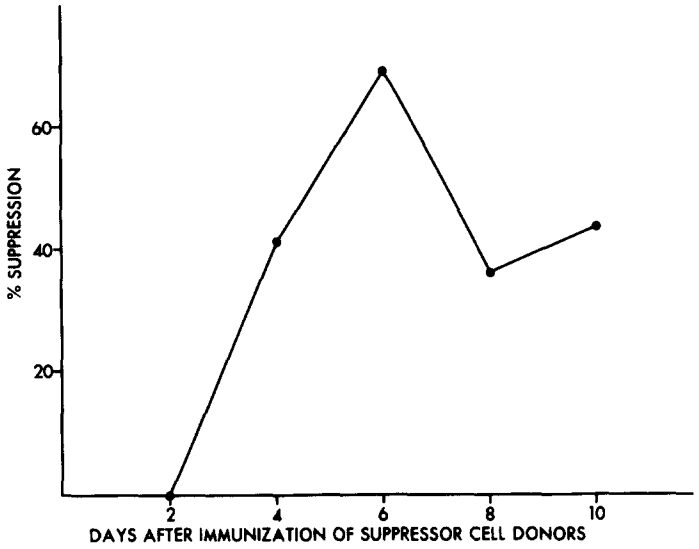

FIG. 1. Ability of different immune cell populations to suppress the primary response to ectromelia. Spleen cells were obtained at various times after infection and injected into recipient mice which had received 2×10^4 PFU attenuated ectromelia virus 1–2 h previously (4×10^7 cells/mouse, i.v.). Recipient spleens were assayed for cytotoxicity 6 days later. Percent suppression values are expressed in relation to percent specific ^{51}Cr release obtained with spleen cells from mice receiving virus only (see footnotes for Table I). Killer to target cell ratio was 25:1.

TABLE III
Specificity of Suppressive Effect

Serum or cells injected*	Percent specific ^{51}Cr release from infected L-929 cells‡	Percent suppression§
None	57.5‖ ± 3.9	0
Normal serum	48.0 ± 1.6	16.0
Normal cells	54.5 ± 0.6	5.2
Ectromelia-immune serum	30.8 ± 3.0	46.4
Ectromelia-immune cells	15.2 ± 1.9	73.6
Listeria-immune serum	49.0 ± 4.0	14.8
Listeria-immune cells	51.0 ± 3.0	11.3

* Experimental procedure as described for Table I. Donor cells and serum were obtained from mice immunized 10 days previously with either 5×10^4 PFU attenuated ectromelia virus or 10^3 *Listeria monocytogenes*.

‡, §, ‖ As for Table I, except that killer to target ratio is 25:1.

injected; increased activity on day 6 may represent the beginnings of a 'memory' or secondary response. The amount of cytotoxic activity present on days 5 and 6, after injection of suppressive cells, was significantly lower than those of the controls in which only virus was injected (Fig. 2).

TABLE IV
Suppressive Effect of Allogeneic and F_1 Hybrid Immune Cells

Immune cells* injected (strain)	Recipient strain	Percent specific ^{51}Cr release from infected target cells‡	Percent suppression§
None	CBA/H	43.7‖ ± 1.0	0
CBA/H	CBA/H	24.2 ± 0.8	45.0
(CBA/H × BALB/c)F_1	CBA/H	36.2 ± 0.3	17.2
BALB/c	CBA/H	41.9 ± 1.0	4.1
A.TL	CBA/H	40.3 ± 0.9	7.8
None	BALB/c	27.5‖ ± 0.9	0
BALB/c	BALB/c	9.1 ± 0.9	66.9
(CBA/H × BALB/c)F_1	BALB/c	22.1 ± 2.7	19.6
CBA/H	BALB/c	26.7 ± 0.6	2.9
A.TL	BALB/c	13.6 ± 1.0	50.5

* Experimental procedure as for Table I. Donor mice were infected with 4×10^3 PFU attenuated ectromelia virus 6 days previously. Donor spleen cells were then injected into recipient mice 1–2 h after injection of 4×10^3 PFU attenuated ectromelia virus (4×10^7 cells per mouse). The *H-2* haplotypes of the mouse strains used are as follows: CBA/H: K^k, IA^k, IB^k, IC^k, S^k, D^k; BALB/c: K^d, IA^d, IB^d, IC^d, S^d, D^d; A. TL: K^s, IA^k, IB^k, IC^k, S^k, D^d.

‡ Appropriate target cells were used: when CBA/H (*H-2*^{k}) were the recipient strain, L-929 (*H-2*^{k}) cells were used; P-815 (*H-2*^{d}) mastocytoma cells served as targets when the recipient strain was BALB/c (*H-2*^{d}). Values are means ± SE of the mean in groups of three wells with spontaneous release subtracted (see Table I). Killer to target ratio was 20:1.

§, ‖ As for Table I.

TABLE V
Effect of Anti-θ Treatment on Suppressive Activity

Cells injected*	Treatment before injection	Percent specific ^{51}Cr release from infected L-929 cells‡	Percent suppression§
None	—	42.6‖ ± 2.0	0
Immune cells	None	10.9 ± 2.2	74.0
Immune cells	NMS	10.4 ± 1.7	76.0
Immune cells	Anti-θ	40.0 ± 3.0	6.0

* Experimental procedure as for Table I. Donor cells were obtained from mice immunized 6 days previously with 2×10^4 PFU attenuated ectromelia virus. Donor cells were then treated with either normal mouse serum (NMS) or anti-θ serum (as described in text) or received no treatment at all; they were then injected into recipient mice (4×10^7 cells/mouse); recipient mice had been immunized 1–2 h previously with 2×10^4 PFU attenuated ectromelia virus.

‡, §, ‖ As for Table I, except that killer to target ratio was 25:1.

Virus Levels in the Spleen after Injection of 6-Day Immune Cells. After the injection of 6-day immune cells 1–2 h after virus, the virus levels in the spleens of recipients were determined after various further time intervals. The injection of 6-day immune cells significantly reduced the amount of virus in the spleen within 24 h and prevented further increase in virus titres (Fig. 3). In contrast,

TABLE VI
Effect of Varying Cell Dose and Virus Dose on Suppressive Activity of 6-Day Immune Spleen Cells

Cells injected per mouse*	Virus dose	Percent specific ^{51}Cr release from infected L-929 cells	Percent suppression‡
	PFU/mouse		
None	2×10^4	42.6‖ ± 2.0	0
10^7	2×10^4	41.6 ± 1.8	2.3
2×10^7	2×10^4	15.8 ± 1.4	62.9
4×10^7	2×10^4	10.9 ± 2.2	74.4
8×10^7	2×10^4	17.8 ± 0.9	58.2
None	8×10^2	46.1 ± 0.6	0
2×10^7	8×10^2	10.3 ± 1.5	77.6
None	4×10^3	49.6 ± 2.0	0
2×10^7	4×10^3	15.1 ± 0.7	69.5
None	2×10^4	52.1 ± 1.1	0
2×10^7	2×10^4	14.6 ± 0.3	72.0

* Experimental procedure as for Table I. Donor cells were from mice immunized 6 days previously with 2×10^4 PFU attenuated ectromelia virus.
‡, §, ‖ As for Table I, except that killer to target ratio was 25:1.

when no cells were injected, virus levels increased to a peak on day 2 and rapidly declined to very low levels by day 5 (Fig. 3).

The Effect of Adult Thymectomy on the Primary Immune Response to Ectromelia. Adult CBA/H mice were either thymectomized or sham-operated and at various times after the operation immunized with ectromelia virus. Cytotoxic activity in the spleen was then assayed 6 or 10 days after immunization. No difference in the level of cytotoxic activity was observed between thymectomized mice and sham-operated controls at either 6 or 10 days after primary immunization (Table VII). It can thus be concluded that adult thymectomy has little effect on the primary immune response to ectromelia infection.

Discussion

T cells active against viral and bacterial infection in mice appear in lymphoid tissues or central lymph within 3 days after infection (10). Since live vaccines are the most effective immunizing agents, the efficient induction of these T cells probably requires antigen presentation in a particular manner after infection of, or processing by, macrophages or, in the case of viruses, infection of other potential 'stimulator' cells (11). Furthermore, recent studies on the infection of mice with lymphocytic choriomeningitis virus (12, 13), ectromelia virus (6, 7, 14), vaccinia virus (15), Sendai virus,[4] and the bacterium *Listeria monocytogenes* (16) suggest that effector T cells in these systems recognize antigenic

[4] Doherty, P. C., and R. M. Zinkernagel. Manuscript in preparation.

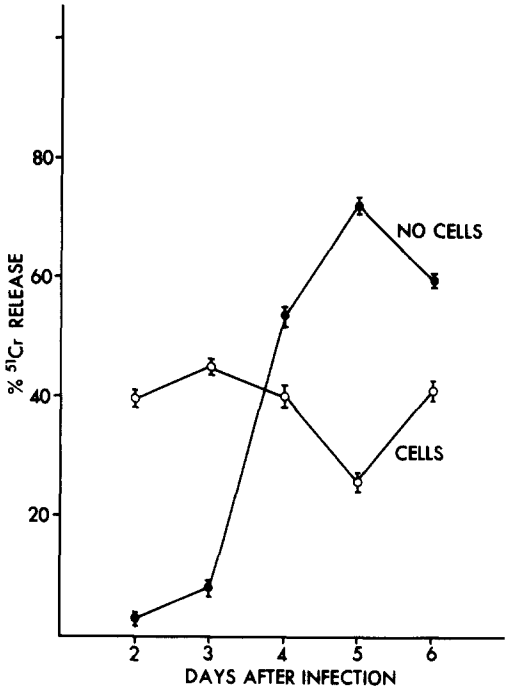

FIG. 2. Kinetics of cytotoxic activity in the spleen after injection of suppressive cells. Recipient mice immunized with 2×10^4 PFU attenuated ectromelia virus received either 5×10^7 6-day immune spleen cells 1–2 h after virus (○—○) or received no further treatment (●—●). Spleens of recipients were then assayed at various further times for cytotoxic activity as described in text. Values are means of three wells with vertical bars representing standard error of the mean. All values are corrected for medium release and killer to target ratio was 60:1.

patterns which are not simply virus-specified or bacterial products alone, but are produced by the action of host cell gene(s) as well as the genome of the infectious agent (11). In the viral systems, the host cell genes required map in the *K* or *D* regions of the *H-2* gene complex (7), i.e., they may be identical to or closely linked to the genes dictating major histocompatibility antigens.

Ectromelia virus multiplies in the spleen for 2–3 days after primary infection by the intravenous route and is then eliminated as the effector T-cell response increases. Effector T-cell activity in the spleen reaches a peak 5–6 days after infection and then declines (4, 10). However, little is known about the regulation of effector T-cell production in the primary response to infection. Regulation can be envisaged to occur at two levels: (*a*) At the stage of induction of precursors of effector T cells, i.e., limitation of the numbers of precursors induced. (*b*) Regulation of effector T-cell proliferation after induction has taken place. In relation to regulation at the level of precursor induction, the simplest regulatory mechanism would be elimination of antigen. The importance of such a mechanism is suggested by the data presented in this report.

In the light of the results obtained it is most likely that the suppressive effect of ectromelia-immune spleen cells and serum on the primary response is due to

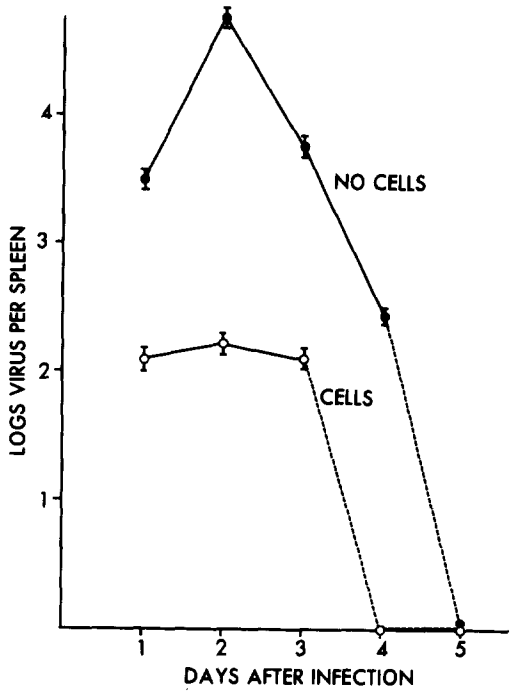

FIG. 3. Virus levels in the spleen after injection of 6-day immune cells. Recipient mice were injected with 2×10^4 PFU attenuated ectromelia virus; 1–2 h later they were injected with either 4×10^7 6-day immune spleen cells (○—○) or received no further treatment (●—●). Spleens of recipients were then assayed for virus content at various further times using a plaque assay on L-929 cells (see text). Values are means of four mice with vertical bars representing standard errors of the mean.

clearance of virus and virus-infected cells, and therefore decreased levels of virus-induced antigenic patterns. The process of viral clearance is triggered by effector T cells. Thus removal of the inductive stimulus necessary for the response seems to be triggered by the effector cells produced by that response, a feedback mechanism of the simplest kind. In support of this conclusion is, firstly, the finding that 6-day immune spleen cells are the most effective suppressors (Fig. 1); this corresponds to the peak of effector activity in vitro and in vivo, i.e., 6-day cells are the most potent killers in an in vitro cytotoxic assay as well as being the most effective in triggering clearance of virus from target organs when injected into infected recipient mice.[3] Secondly, suppression was shown to be anti-θ sensitive (Table V), thus indicating that the active suppressor cell is a T cell or that the mechanism of suppression is T-cell dependent. Thirdly, the suppressive effect, with respect to the dose of immune cells injected, is dose-dependent only up to a certain 'threshold' level (Table VI); it was shown that although 10^7 cells do not suppress the response, strong suppression was produced with 2×10^7 cells and not improved with 4×10^7 or 8×10^7 cells. This suggests that a 'minimum' cell dose of 2×10^7 cells was sufficient to eliminate most of the virus-induced antigen, thereby producing suppression. With respect to virus dose, strong suppression was produced by 2×10^7 cells, irrespective of the dose of

TABLE VII

*The Effect of Adult Thymectomy on the Primary Immune Response to Ectromelia**

Weeks after ATx	Days after immunization	Percent specific ^{51}Cr release from infected L-929 cells‡	
		Sham	ATx
3	6	48.9§ ± 4.1	53.2‡ ± 4.4
	10	3.5 ± 1.5	3.4 ± 1.3
4	6	48.5 ± 4.0	44.6 ± 6.4
	10	0.9 ± 3.6	7.0 ± 4.2
5	6	32.0 ± 3.0	36.9 ± 3.8
	10	13.3 ± 1.0	11.5 ± 1.7
6	6	28.3 ± 1.3	23.8 ± 4.6
	10	10.6 ± 1.7	11.7 ± 0.9
7	6	52.7 ± 1.7	44.9 ± 3.4
	10	17.4 ± 2.7	20.0 ± 2.9
8	6	55.0 ± 3.0	58.5 ± 1.3
	10	16.0 ± 1.7	19.5 ± 2.4

* Adult CBA/H mice (8–9 wk) were either thymectomized or sham operated. At various times after the operation they were immunized with 5×10^4 PFU attenuated ectromelia virus, and spleens were assayed for cytotoxic activity either 6 days or 10 days after immunization as described in text.

‡ As for Table I.

§ Significantly greater release than from uninfected L-929 cells ($P < 0.001$).

virus (Table VI), and it may be argued that this dose of cells was sufficient to remove most of the virus-induced antigen, even at the higher virus doses. Fourthly, virus was barely detectable in the spleens of recipients after injection of immune cells (Fig. 3). Fifthly, for suppression to occur, *D*-region homology at the *H-2* gene complex between donor and recipient mice was sufficient; *I*-region homology alone was neither necessary nor sufficient for suppression (Table IV). Since the major genes required for cytolysis mediated by T cells against ectromelia-infected target cells (7) and T-cell-triggered virus clearance in vivo[2] are located in the *K* or *D* regions of the *H-2* gene complex, this is further evidence for effector T cells being responsible for the observed suppression.

Therefore, the following scheme may be operative: effector T cells in the injected donor population recognize and kill virus-infected cells in target organs (including the spleen). This is followed by monocytic invasion into foci of infection in these target organs (3, 10). This invasion then results in the elimination by phagocytosis and intracellular degradation of much of the virus-induced antigen necessary for the induction of the response, thus resulting in suppression of the primary response as indicated by decreased cytotoxic activity in the spleen 4–6 days later. It was also shown that the suppressive effect was observed when cells or serum were injected 1–2 h or 1 day, but not 2 days, after virus (Table II). This suggests that the sequence of events described above,

which results in a suppression of the primary response, occurs mainly in the first 24–48 h after viral infection and injection of suppressive cells. The clearance of infection in target organs and elimination of the bulk of viral antigen demonstrated by immunofluorescence has been shown to occur in ectromelia virus infection 24 h after injecting immune spleen cells (2, 3). Furthermore, it was recently reported that the injection of syngeneic T cells into irradiated mice after immunization with allogeneic cells suppresses the cell-mediated cytotoxic response against the alloantigens (17). In analogy to the scheme described here, a possible explanation of this result would be as follows: the injected syngeneic T cells would accelerate the cytotoxic T-cell response and consequent rejection of the allogeneic cells (or antigenic stimulus), thus reducing the duration of availability of antigen and resulting in an earlier but lower peak to the cytotoxic T-cell response.

Although virus clearance triggered by T cells is probably the most likely explanation for the observed suppression, the finding that immune serum can also suppress the primary response to a certain extent (Tables I and II) raises the possibility that there are factors produced by B cells in the injected population which prevent induction of cytotoxic T cells. This could occur in a number of ways. Firstly, neutralizing antibody could indirectly reduce the inductive stimulus by limiting further infection of stimulator cells. Secondly, other antibodies may have a blocking effect on induction by either masking antigen on a virus-infected cell or by forming antigen-antibody complexes (with appropriate 'viral' antigens) which blockade T-cell receptors. Such a receptor blockade mechanism by specific immune complexes has been suggested for the response to sheep red blood cells (18). Thirdly, it is possible that antibodies promote monocytic invasion into foci of infection which then removes the inductive stimulus in a manner similar to sensitized T cells (2, 3). It should be pointed out, however, that the nature of suppressive factor(s) in immune serum are unknown and further characterization is necessary.

The previous discussion suggests that removal of antigen plays an important role in the regulation of the primary response at the level of precursor induction. However, other regulatory mechanisms must operate after induction has taken place. Several reports have proposed that two types of T cells are required for the optimal generation of graft versus host reactions in mice (19, 20). One of these types of T cells (called T_1 cells), normally present in the thymus and spleen, may decrease in numbers 2–6 wk after adult thymectomy (20). It is possible that this newly derived T cell is playing a regulatory role (e.g., in the production of suppressor T cells) which is responsible for the decline of the primary response after day 6. If this were the case, the primary response in thymectomized animals, where these T_1 cells are presumably absent, may remain at an elevated level. As was shown, the response of thymectomized mice and sham-operated mice to ectromelia was identical (Table VII). It is thus unlikely that newly-derived T_1 cells play a regulatory role in this system. Alternatively, it is possible that the decline in the primary response is due to suppressive action by a suppressor cell other than T_1 (e.g., a population that is generated with the same kinetics as the effector T cells). A third possibility is that effector cell activity declines because effector cells possess an inbuilt regulatory mechanism which,

after a set number of cell divisions, instructs these cells to dedifferentiate, probably into small lymphocytes which later become 'memory' cells. These possibilities are currently under investigation.

Summary

Spleen cells and serum from mice immunized with ectromelia virus suppressed the immune response to infectious virus when transferred intravenously into recipient mice given an immunizing virus dose. The suppression was reflected in decreased cytotoxic T-cell activity directed against *H-2* compatible virus-infected target cells in the spleens of recipients. Suppression was observed when immune cells or serum were transferred 1–2 h or 1 day after immunization of recipients, but not 2 days after, and was maximal when 6-day immune spleen cells were used as suppressor cells. *H-2* compatibility between donor and recipient mice was necessary for suppression to be expressed. Use of recombinant mice showed that *I*-region compatibility was neither sufficient nor necessary, and that *D*-region compatibility was sufficient. Specificity of suppression was suggested by the finding that cells and serum from mice immunized with *Listeria monocytogenes*, a bacterium, had no suppressive activity on the antiviral response. Anti-θ treatment eliminated the ability of immune cells to suppress, and the suppressive effect was not markedly dose-dependent with respect to both cell dose and virus dose under the conditions employed. Virus levels in the spleens of recipients were significantly reduced after injection of immune cells. Adult thymectomy had no effect on the primary immune response to ectromelia virus infection, thus indicating no role for T_1 cells in the suppressive mechanism.

The results obtained therefore suggested that suppression in this system was due to effector T cells which triggered clearance of virus (and thus, of virus-induced antigens) necessary for the induction of precursors of effector T cells, and that this simple feed-back mechanism normally plays an important role in the regulation of the primary immune response to ectromelia infection at the level of precursor induction. The existence of other postinduction regulatory mechanisms, however, is unknown and under investigation.

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