VIRUS-REPLICATING T CELLS IN THE IMMUNE RESPONSE OF MICE

III. Role of Vesicular Stomatitis Virus-Replicating T Cells in the Antibody Response*

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In the previous papers of this series (1, 2), it was shown that the antigen-reactive T cells capable of replicating vesicular stomatitis virus (VSV)[1] developed in the spleen of mice after the immunization with sheep erythrocytes (SRBC). The time-course of the development of VSV-replicating T cells did not correlate with that of delayed-type hypersensitivity (DTH), helper T cells, or suppressor T cells found in the nylon wool nonadherent (NAd) population. It was also shown that VSV-replicating T cells derived from the T_1 population (Raff and Cantor, [3]), whereas the major part of helper T cells derived from the T_2 population.

Experiments in this paper were undertaken to investigate the function of VSV-replicating T cells in antibody response, taking advantage of the finding that VSV-replicating cells are eliminated by the preinfection procedure (4). Results indicate that VSV-replicating T cells induced by SRBC are nylon wool adherent (Ad) suppressor T cells that develop after the development of helper T cells or nylon wool NAd suppressor T cells. The role of the nylon Ad suppressor T cells in antibody response is discussed in relation to the concept of T_1-T_2 interaction.

Materials and Methods

Methods for the preparation of trinitrophenylated (TNP-) horse erythrocytes (TNP-HRBC) and TNP-SRBC, separation of spleen cells by nylon wool column, preparation of anti-thymocyte serum (ATS), and treatment of cells by ATS and guinea pig serum (C′), in vitro irradiation of cells, assay of anti-TNP antibody producing cells have been described in the previous papers (1, 2).

Mice and Immunization. Male CBA/StMs mice originally obtained from the National Institute of Genetics, Mishima, Japan, 8- to 10-wk old, were used throughout these experiments. Mice were immunized with SRBC by an intravenous injection via tail vein. As a source of dinitrophenyl (DNP)-primed B cells, mice were immunized intraperitoneally with 200 μg DNP-keyhole limpet hemocyanin in Freund's complete adjuvant, and the spleen cells were used 4- to 7-wk after the immunization.

Cell Cultures. Spleen cells were suspended in Eagle's minimal essential medium (MEM) supplemented with 8% heat-inactivated fetal calf serum (FCS) (Flow Laboratories, Inc.,

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[1] *Abbreviations used in this paper:* Ad, adherent; ATS, anti-thymocyte serum; DNP, dinitrophenyl; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; HRBC, horse erythrocytes; MEM, Eagle's minimal essential medium; NAd, nonadherent; PFC, plaque-forming cells; PFU, plaque-forming unit; SRBC, sheep erythrocytes; Tv, the T cells which become VSV-replicating cells in response to antigenic challenge in vitro; VSV, vesicular stomatitis virus.

Rockville, Md.), 2 mM 1-glutamine and 5×10^{-5} M 2-mercaptoethanol. The cell concentration was adjusted to 1×10^7 viable cells/ml. To see the effect of VSV on the in vitro secondary anti-SRBC antibody response, the cells were dispensed in 0.5-ml samples to 16-mm wells of Linbro plastic multidishes (Linbro Chemical Co., New Haven, Conn.), and 5×10^6 SRBC in 0.05-ml samples were added to each well. For the assay of helper activity, a 0.1-ml sample (1 $\times 10^6$ cells) from a helper cell source was mixed with a 0.4-ml sample (4×10^6 cells) from a B-cell source in the well of plastic multidishes, and each well received 5×10^6 TNP-SRBC in a 0.05 ml sample. Normal spleen cells or DNP-primed spleen cells were used as a B-cell source. The plates were incubated at 37°C in a humidified air-7% CO_2 incubator.

Virus. VSV, New Jersey strain, was supplied from the National Institute of Animal Health (Tokyo, Japan). They were grown in L cells and stocked at -70°C after centrifugation to remove cell debris. Rabbit anti-VSV antiserum was kindly supplied by Dr. G. Tokuda (National Institute of Animal Health, Tokyo, Japan).

VSV-Preinfection. Cells were suspended at 5×10^6/ml in 0.5 ml of 5% FCS-MEM containing 5×10^8 plaque-forming units (PFU) of VSV, and incubated at 37°C in a humidified air-7% CO_2 incubator for 2 h. The infected cells were then washed four times to remove free viruses, and dispensed to the culture. In VSV-preinfection experiments, all culture dishes including the control dishes which contained no virus-preinfected cells, received 5 μl (final 1%) of anti-VSV antiserum to prevent the possible secondary effect of free viruses passively introduced or budded from the infected cells.

Results

Augmentation of in Vitro Secondary Anti-SRBC Antibody Response by VSV. Effect of VSV on the secondary antibody response was examined by inoculating VSV into the culture of SRBC-primed spleen cells. Spleen cells were prepared from mice immunized with 10^7 SRBC 3, 4, 7, or 8 days previously, and cultured with SRBC. VSV (10^7 PFU) was added 0, 2, 4, or 5 days (3 h before the cell harvest for the assay) after initiation of culture. Anti-SRBC plaque-forming cells (PFC) were assayed on the 5th-day of culture, and results were expressed as percent of PFC response of each control group receiving no VSV (Fig. 1).

Addition of VSV at the onset of culture resulted in the partial reduction of response regardless of the source of cells. Such reduction may be due to the infection of VSV to macrophages which were shown to be required during the first 1.5 days in the secondary anti-SRBC antibody response in vitro (5).

When VSV was added on the 2nd-day of culture, marked augmentation of the response was observed in the cultures of spleen cells taken 7 or 8 days after immunization (day 7 and day 8 spleen cells, respectively). Considerable augmentation was also seen in day 4 and day 7 spleen cell cultures (cells taken 4 or 7 days after immunization), even if VSV was added on the 4th-day of culture. By contrast, the response of day 3 spleen cells (taken 3 days after immunization) was not enhanced by VSV, regardless of the timing of the inoculation. These results seem to suggest, but do not prove, that VSV may infect and injure a kind of suppressor cell which might begin to develop in the spleen 4 days after and attain the peak 7 days after the immunization.

Interaction between Nylon Wool Column Ad and NAd Spleen Cells from the Carrier (SRBC)-Primed Mice in Anti-Hapten Antibody Response. In the previous paper (2), we showed that VSV-replicating T cells were preferentially found in the nylon Ad fraction of SRBC-primed spleen cells. Then, we examined the activity of the nylon Ad cells in antibody response.

Spleen cells from mice immunized with 10^7 SRBC 7 days previously were separated into nylon Ad and NAd fractions as described in Materials and Methods. Varying
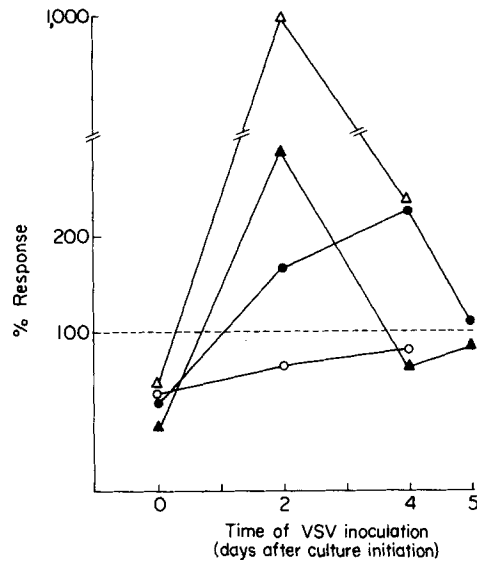
Fig. 1. The augmentation of in vitro secondary anti-SRBC antibody response by VSV. Spleen cells from mice immunized with 10^7 SRBC 3 (○), 4 (●), 7 (△), or 8 days (▲) previously were cultured with SRBC. 10^7 PFU of VSV was added to the culture at the onset, 2, 4, or 5 days later (3 h before the cell harvest). Direct anti-SRBC PFC was determined on the 5th-day of culture. Results are expressed as percent of PFC response of each control culture which received no VSV.
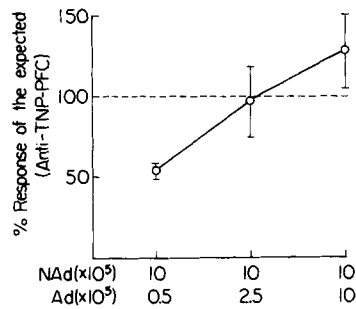

Fig. 2. The interaction between nylon wool NAd and Ad spleen cells from SRBC primed mice in the anti-TNP antibody response in vitro. Spleen cells from mice immunized with 10^7 SRBC 7 days previously were separated by a nylon wool column. 10×10^5 NAd cells were mixed with 0.5, 2.5, or 10×10^5 Ad cells, and then the mixtures were cultured together with 4×10^6 normal spleen cells and 5×10^6 TNP-SRBC. Direct anti-TNP PFC was assayed on the 4th-day. The response was expressed in terms of the percent of the response expected theoretically as the simple summation of the responses by NAd and Ad fractions. Each value and vertical bar represents the mean of the values obtained in three separate experiments and the standard error of the mean.

doses of nylon Ad cells (0.5, 2.5 or 10×10^5) were mixed with 10×10^5 nylon NAd cells, and cultured together with 4×10^6 normal spleen cells and 5×10^6 TNP-SRBC. Direct anti-TNP PFC response was assayed on the 4th-day of culture. In Fig. 2, the mean responses of three separate experiments are shown in terms of percent of the theoretically expected response. By the addition of 0.5×10^5 nylon Ad cells, anti-TNP PFC response was reduced to about 50% of the expected, whereas by the addition of higher numbers of nylon Ad cells the response was augmented, not
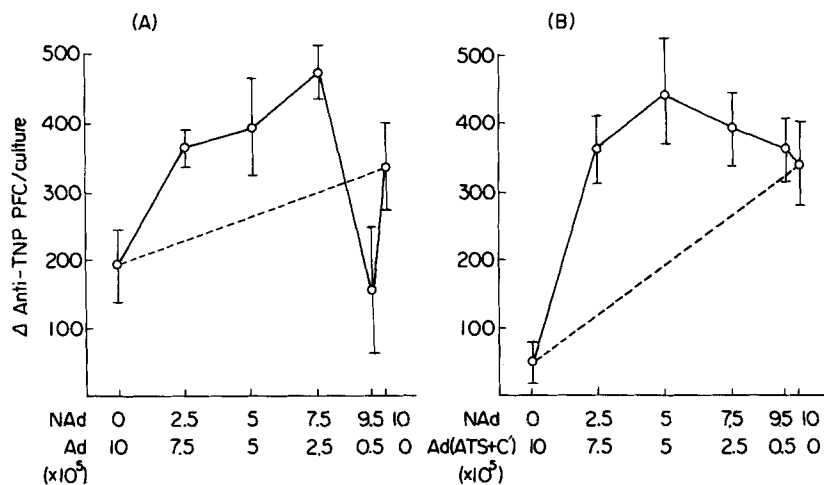
Fig. 3. The suppressor T cells in nylon wool Ad spleen cells. Spleen cells from mice immunized with 10^7 SRBC 7 days previously were separated by a nylon wool column. Ad cells were treated with C′ only or with ATS plus C′ at 37°C for 40 min. NAd cells were mixed with Ad cells treated with C′ only (A) or with ATS plus C′ treated Ad cells (B), at varying ratios, making the total cell number constant (10 × 10^5), and cultured together with 4 × 10^6 DNP-primed spleen cells and 5 × 10^6 TNP-SRBC. Indirect anti-TNP PFC response was assayed on the 4th-day. The mean number of PFC in the cultures which received no SRBC-primed spleen cells was subtracted from each value and thus the results were expressed as Δanti-TNP PFC. The mean and the range of SE for triplicate cultures are shown. The broken line represents the theoretically expected response assuming that no interaction exists between the two cell fractions.

reduced. Although the suppression by small numbers of nylon Ad fraction was not so drastic, the suppression was fully reproducible and significant.

Evidence for the Existence of Suppressor T Cells in the Nylon Ad Fraction of Day 7 Spleen Cells. As shown in the previous paper (2), suppressor T cells found in the nylon NAd fraction of day 3 spleen cells was detectable only when hapten-primed spleen cells were used as a B-cell source. To facilitate the comparison of nylon Ad suppressor cells with nylon NAd suppressor T cells, experiments in this and the following sections were performed mainly with DNP-primed spleen cells as a B-cell source.

Spleen cells from mice immunized with 10^7 SRBC 7 days previously were separated into nylon NAd and Ad fractions. Cells in each fraction were mixed together at varying ratios, making the total number constant (10 × 10^5) to avoid the changes of the culture condition by fluctuating the cell number, and cultured together with 4 × 10^6 DNP-primed spleen cells and 5 × 10^6 TNP-SRBC. After 4 days of culture, indirect anti-TNP PFC response was assayed, and results are shown in Fig. 3 A. As already reported (2), helper activity was always greater in the nylon NAd fraction than in the nylon Ad fraction (compare the value at the extreme right with the value at the extreme left). Mixing the nylon NAd and Ad cells at the ratio of 1:3 to 3:1 resulted in greater anti-TNP PFC response than the value expected as a simple summation of the responses by each fraction. By contrast, mixing at the ratio of 20:1 resulted in a smaller response than expected (55% suppression). Thus it is apparent that the nylon Ad fraction of spleen cells from mice immunized 7 days previously had dual regulatory effect on the helper activity of the NAd fraction of cells.

The effect of ATS plus C′ treatment on the regulatory effect of nylon Ad cells was examined as follows. Nylon Ad cells were treated with ATS plus C′ at 37°C for 40
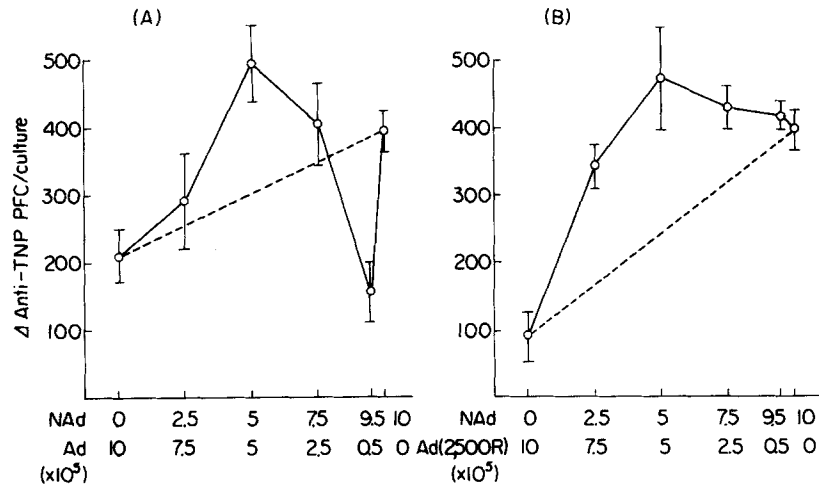
Fig. 4. The abolishment of nylon Ad suppressor cell activity by X-irradiation. Spleen cells from mice immunized with 10^7 SRBC 7 days previously were separated by a nylon wool column. A portion of nylon Ad fraction received 2,500 rads irradiation in vitro. The nylon NAd cells were mixed with unirradiated (A) or irradiated nylon Ad cells (B), making the total number constant (10 $\times$ 10^6). The other experimental protocols and indications are the same as in Fig. 3.

min, washed, and added to the NAd cells at varying ratios. They were cultured together with DNP-primed spleen cells and TNP-SRBC for 4 days. Results are shown in Fig. 3 B. Relatively low helper activity of the nylon Ad fraction was almost completely eliminated by the treatment (compare the value at the extreme left of Fig. 3 A with the value at the extreme left of Fig. 3 B). Suppressive activity was also completely eliminated, suggesting the T-cell nature of the suppressor cells in Ad fraction. Enhancing activity, however, was not affected by ATS plus C' treatment.

Radiosensitivity of Suppressor T Cells in Nylon Ad Fraction. Spleen cells from mice primed with 10^7 SRBC 7 days previously were separated into nylon NAd and Ad fractions. The nylon Ad cells were divided into two parts, of which one part received 2,500 rads irradiation in vitro. Each part was mixed with the nylon NAd fraction at varying ratios, and cultured together with DNP-primed spleen cells and TNP-SRBC for 4 days. Anti-TNP PFC response was shown in Fig. 4. Helper activity in the nylon Ad fraction was partially sensitive to irradiation (compare the value at the extreme left of Fig. 4 A with the value at the extreme left of Fig. 4 B). The suppressive activity manifested at the high NAd: Ad cell ratio (Fig. 4 A) was completely eliminated by the irradiation (Fig. 4 B). On the other hand, the synergistic effect between two fractions at around the 1:1 ratio was resistant to the irradiation.

Absence of Either Suppressive or Enhancing Activity in the Nylon Ad Fraction of Day 3 Spleen Cells. It was shown in the preceding paper (2) that suppressor T cells developed in the spleen 3 days after immunization, and they were found in nylon NAd fraction. Thus, we investigated whether or not the nylon Ad fraction of day 3 spleen cells had suppressive as well as enhancing activity similar to that seen in nylon Ad fraction of day 7 spleen cells. Nylon Ad cells of day 7 spleen, nylon Ad cells of day 3 spleen, or nylon NAd cells of day 3 spleen were mixed at varying ratios with nylon NAd cells of day 7 spleen. The mixtures were cultured together with DNP-primed spleen cells and TNP-SRBC, and anti-TNP PFC response was assayed on the 4th-day of culture. As
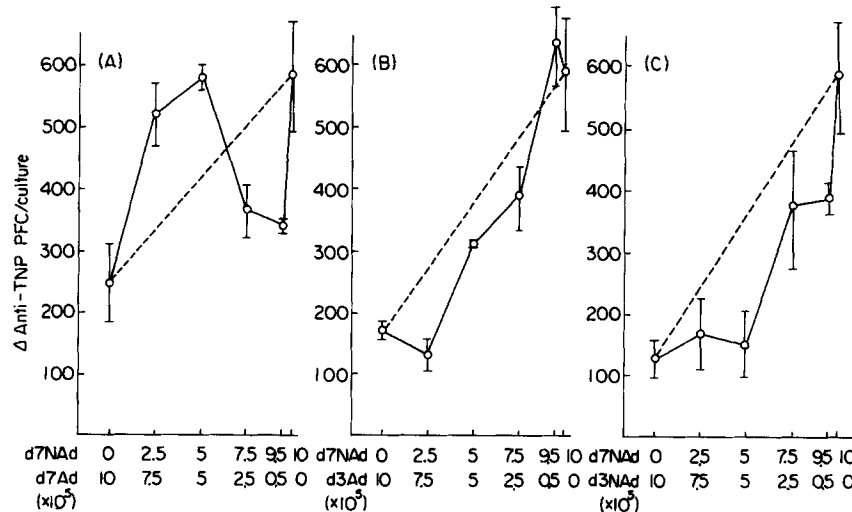
Fig. 5. The delayed development of nylon Ad suppressor T cells compared to nylon NAd suppressor T cells. Spleen cells from mice immunized with 10^7 SRBC 3 or 7 days previously were separated by a nylon wool column. Day 7 (d7) Ad cell (A), day 3 (d3) Ad cells (B), or day 3 NAd cells (C) were mixed with day 7 NAd cells at various ratios, making the total number constant (10 × 10^5). The other experimental protocols and indications are the same as in Fig. 3.

shown in Fig. 5 A, nylon Ad cells of day 7 spleen again showed dual effect on anti-TNP PFC response. On the other hand, such an effect was not observed in nylon Ad cells of day 3 spleen (Fig. 5 B). High concentration of these cells seemed to be suppressive. Conforming to the previous data (2), nylon NAd cells of day 3 spleen suppressed the anti-TNP response maximally at 1:1 ratio (Fig. 5 C).

Abolishment of Nylon Ad Suppressor T Cells by VSV-Preinfection. Among various functional T cells induced by the immunization with SRBC, suppressor T cells in nylon Ad fraction seem thus far to be the best candidates for VSV-replicating T cells. However, there still exists no direct evidence that nylon Ad suppressor T cells are actually capable of replicating VSV on activation by the antigen. Thus, a VSV-preinfection experiment was performed, since it was shown that resting lymphocytes can be latently infected by VSV, and that the activity of lymphocytes which became capable of replicating VSV after the stimulation was eliminated (4).

Spleen cells from mice immunized with 10^7 SRBC 7 days previously were separated into nylon NAd and Ad fractions. Ad cells were infected with VSV at the multiplicity of infection 100 at 37°C for 2 h in a humidified 7% CO_2-air incubator, and then thoroughly washed. These VSV-preinfected or, as a control, uninfected nylon Ad cells were mixed with nylon NAd cells at varying ratios, and cultured together with DNP-primed spleen cells and TNP-SRBC. To eliminate the possible effect of free virus, anti-VSV antiserum was added into the culture throughout the culture period. Anti-TNP PFC was assayed, on the 4th-day. The suppressive effect seen in uninfected nylon Ad cells (Fig. 6 A) was selectively and completely eliminated by VSV-preinfection (Fig. 6 B). On the other hand, a small degree of helper activity in the nylon Ad fraction was scarcely affected by the same treatment (compare the value at the extreme left of Fig. 6 A with the value at the extreme left of Fig. 6 B).

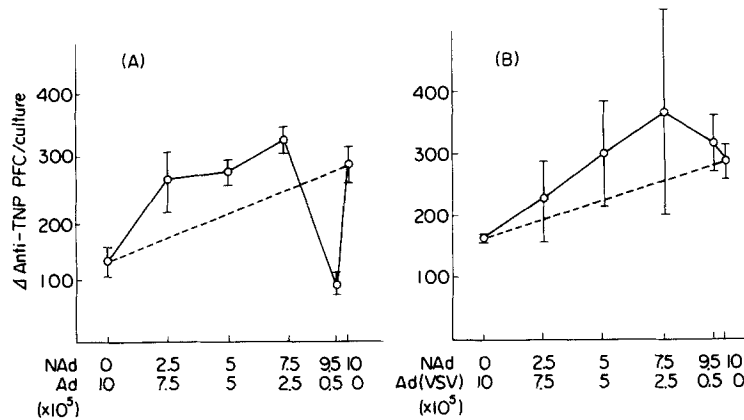*Resistance of the Activities of Helper T Cells and Nylon NAd Suppressor T Cells to VSV-*

Fig. 6.   The abolishment of nylon Ad suppressor T cells by VSV-preinfection. Spleen cells from mice immunized with 10⁷ SRBC 7 days previously were separated by a nylon wool column. A portion of nylon Ad cells were preinfected by VSV in vitro. Nylon NAd cells were mixed with untreated nylon Ad cells (A), or VSV-preinfected nylon Ad cells (B), at varying ratios, making the total number constant. Anti-VSV antiserum (5 µl per dish) was added to all culture dishes at the culture onset. The other experimental protocols and indications are the same as in Fig. 3.

Preinfection. Experiments were designed to investigate the effect of VSV-preinfection on the activities of helper T cells and nylon NAd suppressor T cells. Nylon NAd cells were taken from the spleen of mice immunized with 10⁷ SRBC 3 or 8 days previously, and they were divided into two parts. One part each was preinfected with VSV, and the others were left untreated. Each fraction or various combinations of the fractions was cultured together with DNP-primed spleen cells and TNP-SRBC. Indirect anti-TNP PFC response assayed on the 4th-day of culture is shown in Table I (Exp. I). Helper activity of nylon NAd cells of day 8 spleen was slightly augmented by VSV-preinfection (compare group 2 with group 3), suggesting that the helper T cells were unaffected by VSV-preinfection, and that a small number of VSV-sensitive suppressor cells might be included in this fraction. On the other hand, helper activity of nylon NAd cells of day 3 spleen was not influenced by VSV-preinfection (compare group 4 with group 5). Existence of the suppressor activity in nylon NAd cells of day 3 spleen is evident, since these cells suppressed the helper activity of uninfected as well as VSV-preinfected nylon NAd cells of day 8 spleen (compare group 2 with group 6, and group 3 with group 7). However, as seen in group 8, this suppressor activity was not abolished by VSV-preinfection.

For comparison, the effect of VSV-preinfection on the activity of nylon Ad suppressor cells was also examined. The same source of day 8 spleen cells as used in Exp. I was used in this experiment. Normal spleen cells were used as a B-cell source. As shown in Fig. 6 when DNP-primed B cells were used, suppressor activity of nylon Ad cells was eliminated by VSV-preinfection. Direct anti-TNP PFC response assayed on the 4th-day of culture is shown in Table I (Exp. II). Helper activity in nylon Ad cells was comparable to that in nylon NAd cells in this experiment (groups 2 and 3), and was augmented by VSV-preinfection (group 4). By the addition of a small number of nylon Ad cells to nylon NAd cells (1:20), the response was suppressed (group 5), and the suppressive activity was abolished by VSV-preinfection (group 6).

These results strongly suggest that nylon Ad suppressor T cells replicate VSV

TABLE I

Differential Effect of VSV-Preinfection on the Activities of Helper T Cells and Suppressor T Cells in Nylon NAd and Ad Fractions

Exp.	Group	T-Cell source (× 10⁵)	B-Cell source (4 × 10⁶)	Anti-TNP PFC/culture	
				Direct	Indirect
I	1	None	DNP-Primed		35 ± 7*
	2	d8.NAd(10)	DNP-Primed		356 ± 67
	3	d8.NAd-VSV‡(10)	DNP-Primed		582 ± 96
	4	d3.NAd(10)	DNP-Primed		165 ± 15
	5	d3.NAd-VSV(10)	DNP-Primed		195 ± 57
	6	d8.NAd(10) plus d3.NAd(10)	DNP-Primed		173 ± 48
	7	d8.NAd-VSV(10) plus d3.NAd(10)	DNP-Primed		205 ± 40
	8	d8.NAd(10) plus d3.NAd-VSV(10)	DNP-Primed		149 ± 21
II	1	None	Normal	12 ± 12	
	2	d8.NAd(10)	Normal	327 ± 60	
	3	d8.Ad(10)	Normal	345 ± 54	
	4	d8.Ad-VSV(10)	Normal	661 ± 89	
	5	d8.NAd(10) plus d8.Ad(0.5)	Normal	198 ± 31	
	6	d8.NAd(10) plus d8.Ad-VSV(0.5)	Normal	427 ± 59	

Spleen cells from mice immunized with 10⁷ SRBC 3 days previously (d3) or 8 days previously (d8) were separated by nylon wool column. A portion of nylon wool NAd and Ad cells was preinfected with VSV. Each fraction or various combinations of fractions was cultured together with 4 × 10⁶ DNP-primed spleen cells (Exp. I) or with 4 × 10⁶ normal spleen cells (Exp. II). 5 × 10⁶ TNP-SRBC were added in each culture as the in vitro antigen. All culture dishes received 5 μl of anti-VSV serum at the culture onset. PFC response was assayed on the 4th-day.
* Mean ± SE for triplicate culture.
‡ VSV-preinfected in vitro.

whereas neither helper T cells nor nylon NAd suppressor T cells can replicate VSV on activation by the antigen in vitro.

Discussion

By the inoculation of VSV into the culture, in vitro secondary anti-SRBC antibody response of spleen cells taken more than 3 days after immunization was markedly augmented, whereas no augmentation was observed in the culture of day 3 spleen cells. The highest degree of augmentation was observed in the culture of day 7 spleen cells. It seems possible that interferon induced by VSV in the culture agumented the antibody response. However, the fact that VSV differentially influences the responses of spleen cells taken at various intervals after immunization, makes this possibility unlikely. Moreover, it is shown in the similar experimental systems that the interferon passively introduced into the culture suppresses the response (6, 7). An alternative possibility is that VSV selectively infected and damaged the suppressor cells. This possibility is supported by the experimental results in this paper. If the latter is the case, the result that augmented response was not observed in the culture of day 3 spleen cells but observed in the cultures of spleen cells taken more than 3 days after immunization is compatible with the previous finding that the T cell (Tv) which became a VSV-replicating cell in response to antigenic challenge in vitro began to appear in the spleen 4 days after the immunization (1).

Partial suppression of the response by VSV added at the onset of culture might be due to the damage of macrophages (8) which were shown to be necessary only during the first 1.5 days in the in vitro secondary anti-SRBC response (5). Similar suppression of the in vitro antibody response by Herpes simplex virus, introduced into the culture

during the first 2 days but not later, was reported by Platt-Mills and Ishizaka (9).

The evidence for the existence of two types of supressor T cells in antibody reponse was proposed. In the preceding paper (2), it was shown that suppressor T cells developed in the spleen within 3 days after immunization with 10^7 SRBC. Those suppressor T cells were rich in nylon NAd fraction (Fig. 5), and they were detected only when hapten-primed but not normal spleen cells were used as a B-cell source. The present study, in addition, revealed the existence of another type of suppressor T cells. They were detected in the nylon Ad fraction of day 7 or day 8 spleen cells but not of day 3 spleen cells, and the suppressor activity was expressed regardless of whether the normal or hapten-primed B cells were used. Furthermore, it was shown that the activity of nylon Ad suppressor T cells was manifest only in a very small number, whereas the activity of nylon NAd suppressor T cells was expressed in a dose-dependent fashion.

The expression of the activity of nylon Ad suppressor T cells only with a low dose, but not in high dose, may be explained as follows. In higher doses, the suppressive effect was masked by the augmentation factor(s) concomitantly present in the nylon Ad fraction. Then, the suppressive effect was detected only when the augmentation factor(s) was diluted out. Indeed, the addition of nylon Ad cells to nylon NAd cells in larger numbers resulted in the augmentation of anti-TNP response. This augmentation effect was radioresistant and resistant to ATS plus C' treatment, and thus was supposed to be mediated by non-T cells. Anti-SRBC antibody producing cells rich in nylon adherent fraction seem to be responsible for the augmentation, since it was shown in vitro (1) and also in vivo (10) that exogenously introduced anti-carrier antibody actually enhanced the anti-hapten antibody response. Alternatively, the nylon Ad suppressor T-cell functions only in a small number, and the activity is turned off in larger numbers. Similar paradoxical action of suppressor cells has been reported by others. Haskill and Axelrad (11) showed that blast cell fraction obtained by the sedimentation velocity technique from the SRBC-primed spleen cells suppressed the in vitro secondary anti-SRBC response when added in a small number but failed to suppress in large numbers. Bash et al. (12) also reported a similar phenomenon in the proliferative T-cell response. It is unclear at present which of the alternatives may explain the phenomenon.

Recently, it was shown by Bloom et al. (4) that even resting lymphocytes were infected by VSV and the infection remained latent until the cells were activated by a mitogen. Thus, if preinfected lymphocytes acquire the capacity of VSV replication after the activation by antigen, the function of these cells is expected to be depleted, since replication of VSV results in cell death. Our present experiments with SRBC-primed spleen cells showed that the activity of nylon Ad suppressor T cells was completely eliminated by VSV-preinfection, and that the activity of nylon NAd suppressor T cells was unaffected by the same treatment. These results provided the direct evidence that VSV actually replicated in nylon Ad suppressor T cells but not in nylon NAd suppressor T cells. The data that helper activity of nylon NAd cells of day 3 spleen was not affected by VSV-infection suggest that VSV do not replicate in helper T cells. Thus, the apparent augmentation of the helper activity in both nylon NAd and Ad cells of day 8 spleen may be interpreted to mean that VSV-sensitive suppressor T cells are included in these cell populations and only such suppressor T cells but not helper T cells were damaged by VSV-infection. It may be that the number of VSV-sensitive suppressor T cells included in nylon NAd fraction is only

small, since nylon NAd cells of day 7 or day 8 spleen never expressed the suppressor activity. Because the development of nylon Ad suppressor T cells after immunization coincides with that of Tv (1), it may be said that Tv represents nylon Ad suppressor T cells.

In the antibody response to an erythrocyte antigen, SRBC, it was shown by Araneo et al. (13) that T_1 as well as T_2 population supplied the precursor of helper T cells. On the other hand, Feldmann et al. (14) reported that in the response to a protein antigen, keyhole limpet hemocyanin, T_1 cells acted as amplifier cells in the induction of helper and suppressor T cells from T_2 population, but not as the direct precursors of these cells. Our finding that nylon NAd helper T cells are derived from the T_2 population (2) and nylon Ad helper T cells are from the T_1 population (our unpublished data) is consistent with that by Araneo et al. (13). Furthermore our present results that VSV-replicating T cells suppressed the helper activity of nylon NAd cells suggests the existence of T_1-T_2 interaction also in the antibody response to erythrocyte antigen, since VSV-replicating T cells were shown to belong to T_1 population (2). Our unpublished experiments show that VSV-replicating T cells also suppress the induction of DTH in SRBC system. Thus, it seems that Tv represents the T_1 cells which are preferentially involved in the regulation of the immune response.

Virus selectivity for the lymphocyte subpopulations was reported in different systems using various viruses (Woodruff and Woodruff [15] and Denman et al. [16]). For example Jondal and Klein (17) showed that the receptor for Epstein-Barr virus was present only on B cells but not on T cells of human lymphocytes, whereas Valdimarson et al. (18) reported that the receptor for measles virus was present only on T cells of human lymphocytes. McFarland (19) revealed that helper T cells but not antibody producing B cells were sensitive to the measles virus infection in mice. Furthermore, difference in the sensitivity to murine leukemia virus according to the stages of differentiation of B cells was reported by Cerny and Waner (20). Our present study showed that only a restricted population of T cells permits VSV-replication. Thus, the virus selectivity for lymphocyte subpopulations seems to be based on various steps of virus-lymphocytes interaction, including the distribution of virus receptors, and also the successive stages of viral adsorption, penetration, and replication. Since the selectivity of VSV-replication for T-cell subpopulation does not seem to be based on the distribution of virus receptors (4), the selective replication of VSV in T_1 suppressor cells may reflect the difference of activation process of these cells from that of other T cells.

Summary

The functional role of the T cell (Tv) which can replicate vesicular stomatitis virus (VSV) on activation by the antigen was investigated in antibody response in vitro. By the inoculation of VSV into the culture, marked augmentation of antibody response to sheep erythrocytes (SRBC) was observed in the culture of spleen cells taken more than 3 days after the immunization with SRBC, suggesting that the VSV-susceptible suppressor cells were included in these spleen cells and the activity was eliminated by the effect of VSV.

Development of two distinct types of suppressor T cells was revealed in the spleen of mice after the priming with SRBC. First, nylon wool nonadherent (NAd) suppressor T cells found in the spleen cells taken 3 days after immunization, and second, nylon

wool adherent (Ad) suppressor T cells found in the spleen cells taken ≈ 1 wk after immunization. The activity of nylon Ad suppressor T cells was completely abolished by VSV-preinfection, whereas that of nylon NAd suppressor T cells was unaffected. It was also shown that the helper T-cell activity was not influenced by VSV-preinfection. These results provided direct evidence that nylon Ad suppressor T cell but not nylon NAd suppressor T cell nor helper T cell can actually replicate VSV after the antigenic stimulation. Thus it was strongly suggested that Tv represents the nylon Ad suppressor T cells.

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