

Effect of Antithymocyte Serum on Reaginic Antibody Formation in the Rat

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Summary. The effect of the time and dose of heterologous antithymocyte serum (ATS) on reaginic antibody formation was studied in the rat. Animals were treated with a single intravenous injection of ATS at various times before or after the primary immunization with dinitrophenylated *Ascaris suum* extract (DNP-As) and *Bordetella pertussis* vaccine. If animals were treated with a large lymphopenic dose of ATS shortly before or at the time of immunization, the production of reaginic as well as IgM and IgG antibodies was greatly suppressed, whereas the same treatment if given shortly after the immunization was started significantly enhanced and prolonged the reagin production. On the other hand, smaller doses of ATS even if given at the time of immunization only delayed the reaginic antibody response which also showed a marked prolongation. Furthermore, an unusual sequential production of IgM and IgG antibodies was observed in some of the ATS-treated animals. These results suggest that ATS can inhibit either inductive or regulatory function of the thymus-derived lymphocytes (T cells) depending on the time when it is administered and on the dose of ATS. Some other supporting data indicating that ATS inhibits the T cells specialized in the regulation of antibody formation are also presented.

INTRODUCTION

Antilymphocyte serum (ALS) and antithymocyte serum (ATS) are known to be potent immunosuppressive agents for various immune responses (see Levey and Medawar, 1966; Taub, 1970; Lance, 1970a). Their immunosuppressive activity has been shown to result from direct injury to certain lymphoid cells, especially to thymus-derived lymphocytes (T cells). Accordingly, their activity has been more definitely demonstrated in cell-mediated immunity where T cells play a substantial role in initiation of the response as well as in the tissue injury. Their effect on humoral antibody responses has been reported to be rather variable, depending on antigen dosage (Medawar, 1969; Kerbel and Eiding, 1971; Baum, Lieberman and Frenkel, 1969; Lance, 1970b), the time when ALS was administered (Berenbaum, 1967), and the strain of animals used (James and Milne, 1972). Such inconsistency in the effect of ALS may reflect the diverse role of T cells in humoral antibody responses. Furthermore, Baker, Barth, Stashak and Amsbaugh (1970) reported that administration of ALS potentiated the immune response of mice to pneu-

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mococcal polysaccharide (SSS III), suggesting that ALS may also inhibit the activity of cells specialized in the regulation of antibody response.

Our previous reports (Tada and Okumura, 1971a; Tada, Okumura and Taniguchi, 1972) concerning the regulation of IgE type reaginic antibody formation in the rat suggested that T cells are necessary for the induction of anti-hapten reaginic antibody response, while they also exert an inhibitory effect on the pre-established reagin formation in the later stage of primary antibody response, thus accounting for the quick termination of reaginic antibody response in normal animals. Treatments causing partial depletion of T cells resulted in an enhanced and prolonged reaginic antibody formation (Tada, Taniguchi and Okumura, 1971; Okumura and Tada, 1971a; Taniguchi and Tada, 1971). Moreover, when primed T cells obtained from immunized donors were passively transferred to recipient animals that had been producing reaginic antibody to the same antigen, they exerted a strong inhibitory effect on the reaginic antibody formation of the recipient (Okumura and Tada, 1971b). Since it has been reported that T cells are necessary for production of reaginic antibodies, these results suggest that T cells may have a dual function in the production of reaginic antibodies both as inducer and suppressor.

The present study was undertaken to confirm such regulatory activity of T cells on reaginic antibody response by administration of ATS at various times during the primary and secondary immunization of the rat. It was found that the time and the dose of ATS administration are the crucial factors in determining subsequent suppressed or enhanced antibody responses. The results are discussed in relation to two opposite functions of T cells in the antibody response.

MATERIALS AND METHODS

Animals

Wistar strain adult rats weighing 200 ± 30 grams were used for immunization and the skin test.

Antigen

Dinitrophenylated *Ascaris suum* extract (DNP-As) was prepared by coupling the crude extract of *Ascaris suum* with dinitrophenyl sulphonic acid at an alkaline pH as described in a previous paper (Tada and Okumura, 1971b). The antigen used in the present study contained about 4×10^{-7} moles of DNP per 1 mg of the protein. Dinitrophenylated bovine serum albumin (DNP₂₇-BSA) was prepared by the method of Eisen, Belman and Carsten (1953), and used for titrations of anti-DNP antibodies (see below). *Bordetella pertussis* vaccine containing 3×10^{10} ml of killed organisms was kindly supplied by Dr I. Hashizume of the Chiba Serum Institute, Chiba, Japan.

Anti-thymocyte serum

Two lots of rabbit antisera (Lots 1 and 2) against rat thymocytes were prepared and standardized as follows: rabbits were immunized with 4×10^9 rat thymocytes in Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) via the four footpads, and boosted with the same material in the back muscles on two occasions. The sera were obtained 2 weeks after the third injection. The pooled sera were heated at 56° for 30 minutes, and then were thoroughly absorbed with rat erythrocytes until no haemagglutination was detected. The sera were further absorbed with 1/40 volume of normal rat bone marrow cells.

Two lots of ATS (Lots 1 and 2), prepared as above, were tested for their cytotoxic activity against various lymphoid cells by the dye exclusion test (Takahashi, Old and Boyse, 1970). The sera were serially diluted in Eagle's minimal essential medium (MEM) and mixed with an equal volume of suspensions of freshly prepared thymocytes, spleen cells, lymph node cells and bone marrow cells at a concentration of 10^6 /ml together with 50 CH 50 units/ml of guinea-pig complement. After 30 minutes at 37° , a drop of 1 per cent Trypan Blue was added to each tube, and the percentage of cells including the dye was calculated examining at least 200 nucleated cells. Both lots of ATS used in the present studies maximally killed virtually 100 per cent of the thymocytes, 20 per cent of the spleen cells, 40 per cent of the lymph node cells and < 5 per cent of the bone marrow cells. The highest dilutions of ATS giving 50 per cent cytotoxicity for thymocytes were 1 : 1024 for Lot 1 and 1 : 256 for Lot 2.

Immunization of rats

For reaginic antibody formation, rats were immunized with two successive injections of DNP-As; initially 1 mg with 10^{10} *B. pertussis* vaccine divided among the four footpads on day 0, and 0.5 mg of the antigen alone in the back muscle on day 5. This schedule has proved to be effective for the production of fairly high titres of anti-hapten reaginic antibody in 100 per cent of animals (Tada and Okumura, 1971b), and will be designated as the standard immunization.

To obtain high and persistent reagin formation for cell transfer studies (see below), animals were exposed to 400 R whole body X-irradiation from a 185 KV X-ray machine, and then were immunized by the standard immunization procedure as described above. By this sublethal dose of X-ray before immunization, about 85 per cent of the animals produced enhanced titres of reaginic antibody that persisted for more than 30 days (Tada *et al.*, 1971).

To obtain hyperimmune spleen cells for the cell transfer studies, donor rats were immunized with two intramuscular injections of 1 mg of DNP-As included in Freund's complete adjuvant 2 weeks apart. They were killed 2 weeks after the last injection, and single cell suspensions of their spleens were prepared in Eagle's MEM.

To test the effect of ATS on secondary IgE antibody response, animals were primarily immunized with two injections of 1 mg of DNP-As in Freund's complete adjuvant 2 weeks apart. Four weeks after the onset of primary immunization, they were treated with an intravenous injection of ATS, and then secondarily immunized with 1 mg of DNP-As and 10^{10} pertussis vaccine divided among their four footpads.

Treatment of animals with ATS

To test the effect of ATS on primary reaginic antibody response, groups of five to eight rats were given a single intravenous injection of 2 ml of ATS (Lot 1 or 2), a definitely lymphopenic dose, at various times before or after the immunization was started. For dose-response studies, smaller doses of Lot 1 ATS were given on day 0, immediately before the start of immunization. For the secondary reaginic antibody response, 2 ml of Lot 1 ATS was given intravenously into pre-immunized rats 3 days prior to the secondary immunization.

In vitro treatment of spleen cells with ATS and cell transfer studies

The spleen cell suspension from donors hyperimmunized with DNP-As was treated with

ATS and complement. A suspension of 2×10^9 spleen cells/ml was mixed with an equal volume of 1:20 dilution of ATS (the 100 per cent cytotoxic dose for thymocytes) together with 50 CH_{50} /ml of guinea-pig complement (C), and was incubated for 30 minutes at 37°. As a control, the hyperimmune spleen cells were treated with normal rabbit serum (NRS) plus complement under the same conditions. They were washed twice with chilled MEM and resuspended in the original volume of MEM.

To test the effect of ATS-treated spleen cells on IgE antibody formation, 10^9 cells of the suspension were passively transferred into the recipient rat that had been sublethally X-irradiated and immunized with standard immunization (see above). The cell transfer was made 16 days after the immunization and irradiation of the recipient, at the time when they were continuously producing high titres of reagenic antibody, so as to be able to investigate the suppressive effect of the transferred cells. The detailed experimental procedure has been described elsewhere (Okumura and Tada, 1971b).

Antibody titrations

Reagenic antibody titres against DNP-As were determined of the individual serum obtained at various times after the immunization by the passive cutaneous anaphylaxis (PCA) test (Ovary, 1964). Serial dilutions of the sera were injected intradermally into the shaved backs of normal recipient rats in quantity of 0.05 ml. After 48 hours, animals were challenged with an intravenous injection of 1 mg of DNP-As together with 0.5 ml of 1 per cent Evans Blue. End points showing blueing of more than 5 mm in diameter were determined at the under surface of the skin 30 minutes after the challenge. The PCA titres of the groups on each experimental day were calculated from the geometric mean of \log_2 PCA titres. Hapten specific IgM and IgG antibodies were titrated by the passive haemagglutination test of Stavitsky and Arquilla (1955) using sheep erythrocyte coupled with DNP₂₇-BSA by bisdiazotized benzidine. Haemagglutination titres after treatment of sera with 0.1 M 2-mercaptoethanol (2-ME) were considered to be caused by IgG antibody.

RESULTS

THE EFFECT OF A SINGLE LARGE DOSE OF ATS ON PRIMARY REAGENIC ANTIBODY RESPONSE

The effect of ATS on primary reagenic antibody response was tested by a single intravenous injection of ATS given at various times during the standard immunization. Group 1 was given 2 ml of Lot 1 ATS 3 days before the immunization was started, group 2 on day 0, group 3 on day 5 and group 4 on day 8. This dose was sufficient to cause absolute lymphopenia in peripheral blood. A control group of rats was given 2 ml of normal rabbit serum on day 0.

Fig. 1 shows the kinetics of the reagenic antibody formation of these groups. The control rats given normal rabbit serum showed a transient production of reagenic antibody to DNP determinants in the early stage of immunization, and the kinetics were very similar to those of control responses reported previously (Tada and Okumura, 1971b). However, the pattern of reagenic antibody response was greatly altered by the ATS treatment depending on the time when ATS was administered. As is evident from Fig. 1, the reagenic antibody formation was almost completely suppressed in groups 1 and 2, which had been treated with ATS 3 days before or at the time of the start of immunization. Most of the rats in these groups (1 and 2) produced very little antibody throughout the experimental course. In contrast, groups 3 and 4, which were given the same lymphopenic dose of ATS

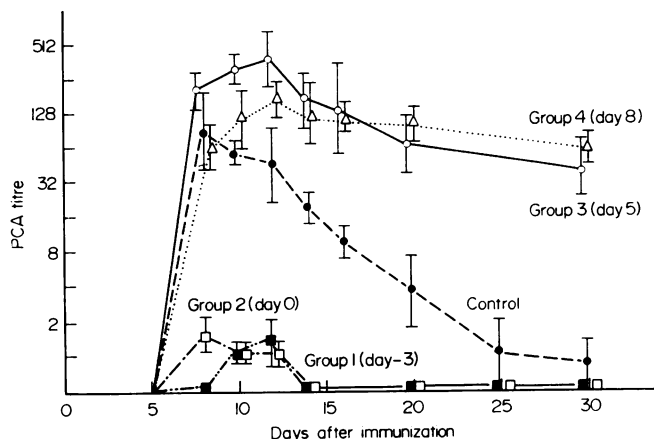


FIG. 1. Kinetics of reaginic antibody formation in the rat given a single large dose (2 ml) of a potent antithymocyte serum (ATS Lot 1) at various times before or after the start of immunization with DNP-As. Note almost complete suppression in groups 1 and 2 given ATS on day -3 and 0, and significant enhancement and prolongation of reagin production in groups 3 and 4 given ATS on day 5 and 8. The control group given no ATS shows a transient production of reaginic antibody. Each point represents the geometric mean with a bar of standard deviation of PCA titres of 5-8 similarly treated rats.

shortly after the start of immunization, showed no suppression but rather enhanced and prolonged production of reaginic antibody. Group 3, treated with ATS 5 days after the start of immunization, reached a higher peak reagin titre than that of the control, and such high titres persisted for a long period of time. The PCA titres of group 4 rats that were given ATS on day 8, at the normal maximum response, were not reduced by the ATS but increased further after the treatment. The PCA titres of groups 3 and 4 were still high on day 30 at the time none of the control rats showed significant reaginic antibody formation. Peak PCA titres and the days of maximum response are shown in Table 1.

A similar result was obtained with another lot of ATS (Lot 2) which had less cytotoxic activity for thymocyte (1:256 for 50 per cent cytotoxicity). Two groups of rat (groups 5 and 6) were treated with 2 ml of ATS before (day -2) or after (day 2) the start of

TABLE 1
EFFECT OF THE TIME AND DOSE OF ATS ON REAGINIC AND HAEMAGGLUTINATING ANTIBODIES AGAINST DNP-As IN THE RAT

Group number (number of animals)	Day of ATS	Dose of ATS (ml)	Maximum† PCA titre (day)	Haemagglutination titre†			
				Day 8		Day 20	
				Bef. 2ME	Aft. 2ME	Bef. 2ME	Aft. 2ME
Control	(5)	0	88 (8)	3200	<20	250	200
1	(8)	-3	1.3 (12)	<20	<20	80	70
2	(8)	0	1.5 (8)	60	<20	<20	<20
3	(8)	+5	161 (12)	2200	540	200	160
4	(8)	+8	362 (12)	3400	<20	110	100
5*	(5)	-2	8 (16)	227	<20	330	100
6*	(5)	+2	295 (16)	2560	533	640	180
7	(6)	0	64 (25)	3627	160	10240	640
8	(6)	0	108 (20)	7761	320	20480	211

* Groups given Lot 2 ATS.

† Geometric means of reciprocal antibody titres of the groups.

immunization. As depicted in Table 1, the treatment before immunization was strongly suppressive for reagin synthesis, though not complete as with Lot 1, whereas that given after the start of immunization was significantly enhancing.

The passive haemagglutination (HA) test was performed to investigate the effect of ATS on anti-hapten IgM and IgG antibody formation. Table 1 also shows HA titres of these groups on days 8 and 20, since these days have been shown to represent maximum responses in early IgM and late IgG antibodies (Tada and Okumura, 1971b). In groups 1, 2 and 5, which were given ATS before or at the time of the start of immunization, both early IgM and late IgG antibody responses were severely suppressed, whereas in groups 3 and 6 which were given ATS shortly after the immunization was started, a premature appearance of IgG antibody was observed on day 8, at the time when none of the control rats produced 2-ME-resistant antibody.

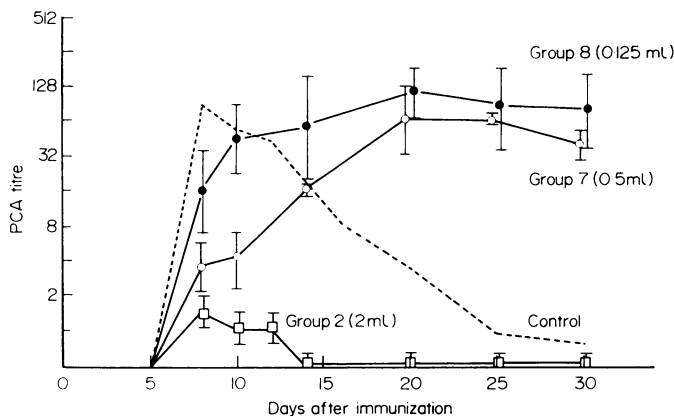


FIG. 2. Kinetics of reagin antibody formation in the rat given various doses of ATS (Lot 1) on day 0. Note the significant suppression of reagin formation in group 2 given a large dose (2 ml), while groups 7 and 8 given smaller doses (0.5 and 0.125 ml) show the delayed but persistent reagin antibody formation as compared to that of the control.

THE EFFECT OF DOSE OF ATS

Since the effect of ATS has been shown to be dose-dependent, dose effect of ATS on reagin antibody response was examined. Two groups of animals (groups 7 and 8) were treated with 0.5 ml and 0.125 ml of the potent ATS (Lot 1) on day 0, at the time when the larger dose (2 ml) was almost completely inhibitory for reagin production.

Fig. 2 shows the kinetics of reagin antibody formation in these low dose groups in comparison with those of the high dose and the control groups. In both low dose groups, reagin antibody response was delayed but in no case was it diminished. Moderately high titres persisted for a longer period of time than in the controls. Although there was no strict dose-response relationship, the over-all reagin antibody response in group 8, given 0.125 ml, was better than in group 7 given 0.5 ml, suggesting that the magnitude of reagin response was inversely related to the degree of damage to T cells (Table 1).

Of more interest are the HA-titres in these low dose groups. As shown in Table 1, high titres of 2-ME sensitive antibody were detected in both groups on day 20 when the control animals showed only 2-ME-resistant IgG antibody, indicating that a moderate dose of

ATS would prolong not only IgE antibody but also IgM antibody formation. In these two groups an earlier appearance of IgG antibody on day 8 was also observed.

SUSCEPTIBILITY OF SUPPRESSOR LYMPHOCYTES TO ATS TREATMENT *in vitro*

Our previous report described that passively transferred spleen and thymus lymphoid cells obtained from rats hyperimmunized with DNP-As exerted a strong suppressive effect on an ongoing reaginic antibody response against this hapten (Okumura and Tada 1971b). This suppressive effect was considered to be due to the presence of specific suppressor cells in the hyperimmune thymus and spleen cell preparations. In order to confirm that such suppressor lymphocytes actually belong to the ATS-sensitive T-cell population, the spleen cells having the suppressor activity for reagin formation were treated with ATS and complement and then were transferred to the reagin-producing recipient.

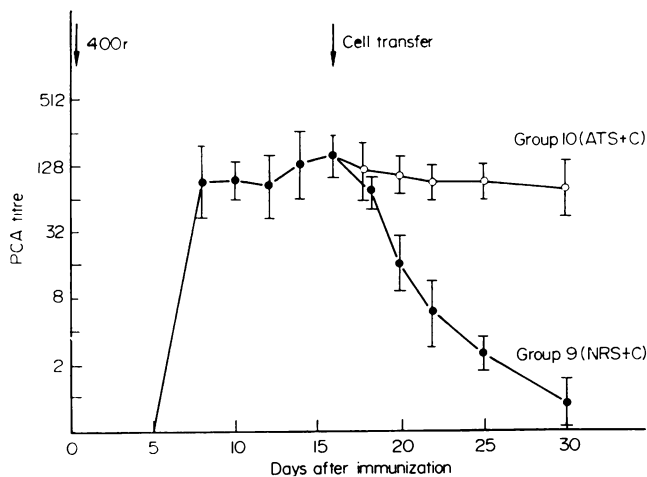


FIG. 3. Abrogation of suppressor activity of hyperimmune spleen cells by *in vitro* treatment with ATS and complement (group 10). The spleen cells treated with normal rabbit serum and complement preserve the strong suppressor activity on the ongoing reaginic antibody formation in the recipient (group 9).

Groups of animals were exposed to 400 R of X-irradiation and were immunized with DNP-As and pertussis vaccine so as to produce high and persistent reaginic antibody. Spleen cells from DNP-As-hyperimmunized donors containing suppressor activity were treated either with ATS or with NRS in the presence of guinea-pig complement at 37°. After incubation for 30 minutes the cells were washed and 10^9 nucleated cells were passively transferred to the irradiated reagin-producing animals on day 16, at the time when they were continuously producing high titres of reaginic antibody.

The results of this experiment are shown in Fig. 3 and Table 2. The passive transfer of hyperimmune spleen cells treated with NRS and complement caused a drastic decrease of PCA titres within a few days after the cell transfer (group 9), whereas the treatment with ATS and complement completely abrogated such inhibitory activity of the spleen cells (group 10). Since the half life ($t_{1/2}$) of rat IgE antibody in the serum is only 12 hours (Okumura and Tada, unpublished data), the rapid decrease of PCA titre in group 9 reflects the rapid termination of reagin synthesis caused by suppressor T cells, which were

TABLE 2
EFFECT OF *in vitro* TREATMENT WITH ATS AND COMPLEMENT ON ACTIVITY OF HYPERIMMUNE SPLEEN CELLS

Group number	Cells* treated with	PCA titres of the recipient†		
		Day 16	Day 25	Percentage decrease
9	NRS+C	181	2.3	98.7
10	ATS+C	147	84	42.9

* 1×10^9 of treated spleen cells were passively transferred to reagin-producing recipients on day 16.

† Geometric mean PCA titres before (day 16) and 9 days after (day 25) the cell transfer.

shown to be eliminated by ATS and complement. In this particular experiment, animals had been exposed to X-irradiation, resulting in haemagglutination titres too low to demonstrate any effect of hyperimmune spleen cells on IgG and IgM antibody formation.

EFFECT OF ATS ON SECONDARY REAGINIC ANTIBODY RESPONSE

The above studies indicate that ATS inhibits the function of T cells that negatively regulate reagin formation. As it has been shown that secondary reaginic antibody formation is hardly elicited by repeated immunization, especially with Freund's complete adjuvant (Mota, 1964; Binaghi and Benacerraf, 1964; Tada, Okumura and Taniguchi, 1972), it seems possible that the large number of inhibitory T cells generated by the primary

TABLE 3
EFFECT OF ATS ON SECONDARY REAGINIC AND HAEMAGGLUTINATING ANTIBODY RESPONSE AGAINST DNP-As IN PRE-IMMUNIZED RATS

Group number (number of animals)	Treated* with	Maximum† PCA titre	Haemagglutination titre†	
			Bef. 2ME	Aft. 2ME
11 (6)	NRS	0	905	905
12 (5)	ATS	11	640	640

* Two millilitres of NRS or ATS were given intraperitoneally 3 days before the secondary immunization with DNP-As and pertussis vaccine.

† Geometric means of reciprocal antibody titres obtained 14 days after the secondary immunization.

immunization have prevented the induction of secondary reaginic antibody response. Therefore, we tested the effect of ATS on secondary reaginic antibody response by injecting ATS 3 days prior to the secondary immunization with DNP-As and pertussis vaccine in the rat, which had previously been immunized with the same antigen in Freund's complete adjuvant.

Groups of rats were immunized with two injections of 1 mg of DNP-As in Freund's complete adjuvant 2 weeks apart. Two weeks after the second injection, they were treated either with 2 ml of NRS (Group 11) or Lot 1 ATS (Group 12) intravenously, and 3 days later they were secondarily immunized with 1 mg of DNP-As and pertussis vaccine divided among the four footpads. Reaginic antibody formation was likewise tested by the PCA test after the secondary immunization.

As shown in Table 3, all of the animals pre-immunized with DNP-As and given no ATS (Group 11) could not produce reaginic antibody at all upon subsequent secondary immunization with DNP-As and pertussis vaccine, whereas all of the rats given a lymphopenic dose of ATS before secondary immunization (Group 12) produced low titres of reagin against the immunizing antigen. However, no significant difference in titres of haemagglutinating antibody that was almost entirely 2-ME-resistant at this stage of immunization was detected between these two groups.

DISCUSSION

The present observations indicated that ATS would exert two opposite effects on the reaginic antibody formation against a hapten-carrier conjugate, mainly depending on the time when it was administered and on the dose of ATS. The induction of the reaginic antibody response, as well as other classes of antibody responses, was severely suppressed by the administration of a single large dose of ATS before or at the time of start of immunization, whereas the same treatment given shortly after the immunization was started was distinctly enhancing for reagin formation. Smaller doses of ATS, which might have caused relative depletion of T cells, only delayed the reaginic antibody response and rather prolonged the production of reagin even if they were given on day 0 at the time when the large dose was significantly inhibitory. These time-dose-dependent opposite effects of ATS seem to reflect the dual function of T cells in the regulation of reaginic antibody formation, which has been suggested in a previous report (Tada, Okumura, Taniguchi, 1972). It has been shown that T cells had to be primed with the carrier determinants prior to the actual synthesis of anti-hapten reaginic antibody, indicating that helper T cells were required for reagin synthesis by B cells (Tada and Okumura 1971a). However, the reaginic antibody response thus developed was very transient in the early stage of primary immunization, and the secondary reaginic antibody response was found hardly to occur. The mechanism which accounts for this early termination of reaginic antibody response was suggested by the experiment in which passively transferred spleen and thymus cells from donors hyperimmunized with DNP-As or *Ascaris* extract exerted strong suppressive effect on the ongoing anti-DNP IgE antibody formation of the recipient (Okumura and Tada, 1971b). Thus it has been suggested that T cells primed with the carrier molecule of the antigen would first assist in the induction of hapten-specific reaginic antibody formation by B cells, but they later may negatively regulate the pre-established reagin formation, accounting for the transient nature of this type of antibody response.

It is probable from the present observations that ATS given before or at the initial stage of primary immunization may have depleted the helper T cells causing the suppression of all classes of antibody formation. On the other hand, ATS given after the inductive cell interaction between helper T cells and reagin-forming B cells has taken place would then inhibit the terminating process by suppressor T cells, thus causing the observed enhancement and prolongation of reagin synthesis. This idea is consistent with our previous findings that neonatal thymectomy caused complete inhibition of reagin formation, while adult thymectomy which may cause relative depletion of T cells resulted in an enhanced and prolonged production of reaginic antibody (Okumura and Tada, 1971a). It has also been shown that sublethal whole body X-irradiation, as was used in the present cell transfer study, and treatments with certain immunosuppressive drugs enhance the primary reaginic antibody response if administered at appropriate times (Tada *et al.*, 1971;

Taniguchi and Tada 1971; Tada, Okumura, Ochiai and Iwasa 1972). White and Holm (1973) recently described that ALS potentiated the rat reagenic antibody formation against associated form of keyhole limpet haemocyanin (KLH) which is rather a weak antigen for reagin production in normal rats. Reagenic antibody formation against horse serum constituents in mice treated with horse anti-mouse lymphocyte serum has also been reported by Kind and Ako (1969). The smaller doses of ATS, which were not completely suppressive for the induction of reagenic antibody response even though given on day 0, were nevertheless inhibitory for the terminating process resulting in a long duration of reagin synthesis. These observations suggest that such treatments have preferentially impaired the suppressive regulatory function of T cells resulting in the observed long duration of enhanced reagin formation.

This was further supported by the present cell transfer experiment in which the suppressor spleen cells were treated *in vitro* with ATS and complement. By this treatment the suppressor effect of hyperimmune spleen cells was completely abrogated, indicating that ATS-sensitive cells actually participate in the regulation of reagenic antibody formation. Such effect of ATS was also visualized *in vivo* by treating the pre-immunized animals with ATS. The pre-immunized animals did produce reagenic antibody upon subsequent secondary immunization with the same antigen if they were treated with ATS prior to the second injection of the antigen, while none of the NRS-treated pre-immunized animals produced secondary reagenic antibody response at all. All of these results are consistent with the idea that T cells have dual function as amplifier and suppressor, and ATS may inhibit either one of the functions depending on the time and the dose of its administration. This concept may at least partially explain the previously conflicting effect of ATS on humoral antibody responses observed by several authors (Baum *et al.*, 1969; Baker *et al.*, 1970; Kerbel and Eidinger, 1971; Berenbaum, 1967).

Furthermore, it is of interest that ATS given after the immunization had started caused a premature appearance of IgG antibody. This observation certainly argues against the idea that the major mechanism which turns off reagin synthesis is the 'feed-back' regulation by serum IgG antibody, although this would be operative in certain experimental conditions (Tada and Okumura, 1971b; Strannegård and Belin, 1970). Moreover, the treatment with lower doses of ATS given on day 0 caused an enhancement and prolongation of IgM antibody synthesis in addition to the prolonged IgE antibody formation. Barthold, Stashak, Amsbaugh, Prescott and Baker (1973) mentioned in their recent report that IgG and IgA antibody-forming cells against SSS III are also increased by ALS treatment in certain strains of mice. All of these findings suggest that T cells may participate in the homeostatic control mechanisms involved in the determination of the magnitude as well as the sequence of antibody formation belonging to various immunoglobulin classes, and that ATS treatments causes disturbance of such regulatory mechanisms resulting in the unusual sequential production of antibodies. Indeed, it has been suggested that T-cell requirement differs in antibody responses of different classes of immunoglobulins (Mitchell, Mishell and Herzenberg, 1971; Taylor and Wortsis, 1968; Tada *et al.*, 1972).

The mechanisms with which T cells regulate the function of antibody-forming B cells are unknown. Our recent histological observations (Ochiai, Okumura, Tada and Iwasa 1972; Tada, 1973) demonstrated that rats treated with ATS or with lymphocytosis-promoting factor of *B. pertussis*, which also causes T-cell depletion in the thymus and the thymus-dependent areas of lymphatic tissues, exhibited a burst of proliferation of plasma cells and follicular lymphocytes, suggesting that T cells normally control the proliferation

and differentiation of B cells. Although it is still uncertain at the present time whether helper T cells and suppressor T cells are identical or not, it is of interest that both functions are susceptible to ATS treatments. Thus it seems probable that the mode of antibody responses is definitely related to the delicate balance of T and B cells, changes in which sharply influence the magnitude and sequence of different antibody responses.

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