# PRESENCE OF RECEPTORS FOR ANTIGEN AND ANTIBODY ON DENGUE VIRUS-INDUCED SUPPRESSOR T CELLS AND THEIR PRODUCTS

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## ABSTRACT

Dengue type 2 virus (DV)-induced suppressor pathway consists of a cascade of $Ts_1$ cell and its product SF, $Ts_2$ cell and its product $SF_2$ and the $Ts_3$ cell which mediates antigen-specific suppression of humoral immune response. The present study was undertaken to investigate if the different constituents of this pathway have complementary receptors. It was observed that the suppressor activities of $Ts_1$, SF and $Ts_3$ were abrogated by binding with anti-DV antibody while those of $Ts_2$ and $SF_2$ were abrogated by binding with DV antigen. Thus, DV-induced suppressor pathway has a network of idiotype-anti-idiotype-like interactions which maintain the antigen specificity.

## INTRODUCTION

DENGUE type 2 virus (DV) induces a suppressor pathway in mice involving three generations of suppressor T cells which suppress antigen-specific antibody forming cells. DV induces production of a population of suppressor T cells ($Ts_1$) in mouse spleen. $Ts_1$ produce a soluble suppressor factor (SF) which is transmitted via macrophages to recruit a second set of suppressor T cells ($Ts_2$). The latter produce a prostaglandin-like suppressor factor ($SF_2$) which recruits the third sub-population of suppressor T cells ($Ts_3$) to mediate antigen-specific suppression of humoral immune response through inhibition of helper T cell generation and direct inhibition of B cell functions[1–3].

Similar suppressor cell pathways consisting of multiple cellular components, including macrophage-like cells and subpopulations of T cells, have been described in a number of models using synthetic antigens. The signal between different subpopulations of suppressor T cells is transmitted by soluble factors produced by them[4,5]. In such cascades there is sequential induction of steps — the earlier one inducing the next — and this may result in maintenance of specificity of function throughout the pathway. The network hypothesis of Jerne[6] visualizes the interactions of different types of cells and their products in the immune response as being mediated by complementary receptors on the cell surfaces and on the soluble products — idiotypic-anti-idiotypic interactions. Thus, according to the general scheme, if $Ts_1$ expresses the anti-idiotype or idiotype; it can bind the antigen[5] and immunoglobulins[7] and may express idiotypic determinants similar to the dominant idiotypes characteristic of antibodies against the inducing antigens[8]. In the present study, therefore, an effort was made to examine the binding of DV-specific-antigen and anti-DV-antibody to the different subpopulations of the DV-induced suppressor T cells and their products to know if they have complementary receptors. The findings of the present study fit well in the general network scheme where $Ts_1$, SF and $Ts_3$ have similar receptors while $Ts_2$ and $SF_2$ have the complementary sites.

## MATERIALS AND METHODS

Studies were carried out on 3–4-month-old inbred Swiss albino mice. Dengue type 2 virus, strain P23085, was used. A suspension of DV-infected adult mouse brain was used directly, as described elsewhere[9].

*Preparation of $Ts_1$ cells and SF*

Preparation of $Ts_1$ cells and SF has been described earlier[10]. In short, spleens were collected from sick moribund mice given $10^3$ $LD_{50}$ of DV (i.c.) 8–11 days earlier. A cell suspension was prepared in Earle's minimum essential medium (MEM) containing 10% FCS. This suspension was used as $Ts_1$ cells. A 10% (w/v) homogenate of infected spleens was prepared in phosphate-buffered saline (PBS) pH 7 using MSE tissue homogenizer. The homogenate was centrifuged at 2000 *g* for 10 min at 4°C to remove cell debris, then at 103,500 *g* for 3 h. The supernatant was used as SF and stored at −20°C.

*Preparation of $Ts_2$ cells and $SF_2$*

Preparation of $Ts_2$ cells and $SF_2$ has been described earlier[10]. In brief, normal mouse spleen cells ($1 \times 10^7$ cells/ml) were treated with 0.4 ml ($10^{-2}$ dilution) of SF at 37°C for 1 h. The cells were washed thrice, cultured for 24 h and then centrifuged at 2000 *g* for 10 min to separate the cells from the fluid. The cells were resuspended in the medium and used as $Ts_2$ cells and the fluid was used as $SF_2$.

*Preparation of $Ts_3$ cells*

$SF_2$ induces the normal mouse spleen T lymphocytes to produce $Ts_3$ cells[10]. Briefly, $1 \times 10^7$ normal spleen cells treated with 0.4 ml (undiluted) of $SF_2$ for 1 h at 37°C were washed thrice, suspended in MEM-HEPES containing 10% FCS and cultured for 24 h at 37°C in presence of 5% $CO_2$. The cultures were then harvested and centrifuged at 2000 *g* for 10 min at 4°C. Cells were resuspended in MEM and used as $Ts_3$ cells.

*Assay of suppressor activity*

The suppressor activity of various suppressor cells and their products was assayed *in vivo*. Mice primed with $10^3$ $LD_{50}$ of DV (i.p.) were given 48 h later $5 \times 10^6$ variously treated suppressor cells or 0.2 ml of suppressor factor intravenously. On the seventh day of the virus inoculation, spleens were collected and DV-specific direct antibody plaque forming cells (PFC) were counted by the haemolysis-in-gel technique of Jerne and Nordin[11] as described elsewhere[12]. From each mouse spleen multiple slides were prepared. Mean value ± SD of the data obtained from repeated experiments are presented after deducting background PFC.

## RESULTS

*Effect of treatment of suppressor cells with DV antigen*

Infected mouse brain was used as a source of DV and its antigen preparation; it was therefore used as an antigen for treatment of the suppressor cells. Different subpopulations of the suppressor T cells prepared from mouse spleen were treated with different dilutions ($10^{-1}$ to $10^{-4}$) of the DV-infected mouse brain suspension at 4°C for 1 h. The cells were washed and their suppressor activity was assayed. For

**Table 1** *Activity of suppressor cells treated with DV-antigen*

| | $Ts_1$ | | $Ts_2$ | | $Ts_3$ | |
|---|---|---|---|---|---|---|
| Dilutions of DVMB | PFC | % Suppression | PFC | % Suppression | PFC | % Suppression |
| $10^{-1}$ | 317 ± 70 | 57 | 685 ± 29 | 10 | 355 ± 24 | 51 |
| $10^{-2}$ | 369 ± 61 | 50 | 683 ± 31 | 10 | 354 ± 46 | 51 |
| $10^{-3}$ | 352 ± 57 | 52 | 507 ± 44 | 33 | 365 ± 35 | 50 |
| $10^{-4}$ | 365 ± 50 | 51 | 427 ± 45 | 44 | 370 ± 38 | 49 |
| Untreated | 333 ± 73 | 55 | 410 ± 26 | 46 | 388 ± 26 | 46 |
| Without cells | 736 ± 54 | 0 | 757 ± 27 | 0 | 723 ± 44 | 0 |

DVMB–DV-infected mouse brain tissue homogenate; PFC–Count/$2 \times 10^6$ spleen cells.

control, the cells were treated with similar dilutions of normal mouse brain suspension.

The data presented in table 1 show that the suppressor activity of $Ts_1$ and $Ts_3$ cells was not affected by pretreatment with DV-infected brain antigen. In contrast, the suppressor activity of the $Ts_2$ cells was abrogated following treatment with DV-infected mouse brain, the abrogation of the suppression being dose-dependent (table 1). Treatment with normal mouse brain suspension had no effect on the activity of the different subpopulations of the suppressor cells.

To facilitate presentation the data from the experiments with normal mouse brain suspension is not shown in table 1.

*Effect of treatment of suppressor factors with DV antigen*

DV-infected adult mouse brain was grinded in 3.6 ml MEM without serum containing SF ($10^{-3}$ dilution) or $SF_2$ ($10^{-1}$ dilution) and was incubated at 4°C for 1 h. After centrifugation at 2000 *g* for 10 min the clear supernatant was collected and ten-fold dilutions were prepared and their suppressor activity assayed. For control $SF/SF_2$ was similarly treated with normal mouse brain. The findings were compared with those of untreated preparations. The data presented in table 2 show absence of any reduction in the titre of SF following adsorption of DV-infected or normal mouse brain. On the other hand, the suppressor activity of $SF_2$ ceased completely following treatment with DV-infected mouse brain.

*Effect of treatment of suppressor cells with anti-DV-antisera*

Different subpopulations of the suppressor cells were treated with anti-DV-antisera, using antibody in excess, or normal serum at 37°C for 1 h. The cells were washed and their suppressor activity was assayed. The findings presented in table 3 show that treatment of $Ts_2$ cells with anti-DV-antisera had no effect on their suppressor activity which was 46–50%. On the other hand, the suppressor activity of $Ts_1$ and $Ts_3$ cells was reduced to 12–13% from 52 to 58% by treatment with anti-DV-antisera.

*Effect of adsorption of SF and $SF_2$ on antibody columns*

Immunosorbant columns were prepared by coupling anti-DV-antisera with the cyanogen bromide activated sepharose 4B. For control, the columns were prepared using normal mouse serum. The preparations of SF and $SF_2$ obtained after adsorption on the columns were assayed for suppressor activity. The data presented in table 4 show that suppressor activity

**Table 2** *Activity of suppressor factors treated with DV antigen*

| | Unadsorbed | | Adsorbed with DVMB | | Adsorbed with NMB | |
|---|---|---|---|---|---|---|
| Dilutions of $SF/SF_2$ | PFC | % Suppression | PFC | % Suppression | PFC | % Suppression |
| SF | | | | | | |
| $10^{-3}$ | 460 ± 30 | 37 | 515 ± 16 | 34 | 430 ± 25 | 36 |
| $10^{-4}$ | 533 ± 25 | 27 | 556 ± 14 | 29 | 499 ± 16 | 26 |
| $10^{-5}$ | 588 ± 27 | 19 | 597 ± 13 | 24 | 584 ± 28 | 13 |
| Nil (Control) | 727 ± 35 | 0 | 785 ± 33 | 0 | 670 ± 37 | 0 |
| $SF_2$ | | | | | | |
| $10^{-1}$ | 405 ± 28 | 45 | 692 ± 37 | 9 | 427 ± 18 | 38 |
| $10^{-2}$ | 493 ± 22 | 33 | 684 ± 66 | 10 | 492 ± 20 | 28 |
| $10^{-3}$ | 575 ± 32 | 22 | 703 ± 80 | 7 | 586 ± 38 | 14 |
| Nil (Control) | 734 ± 49 | 0 | 756 ± 38 | 0 | 684 ± 47 | 0 |

DVMB–DV-infected mouse brain; NMB–normal mouse brain; PFC–Count/$2 \times 10^6$ spleen cells.

**Table 3** *Activity of the suppressor cells treated with anti-DV-antisera*

| Treatment of cells | $Ts_1$ PFC | $Ts_1$ % Suppression | $Ts_2$ PFC | $Ts_2$ % Suppression | $Ts_3$ PFC | $Ts_3$ % Suppression |
|---|---|---|---|---|---|---|
| Anti-DV-antisera | 606 ± 49 | 12 | 367 ± 53 | 50 | 680 ± 45 | 13 |
| Normal sera | 367 ± 66 | 47 | 363 ± 77 | 50 | 382 ± 62 | 51 |
| Untreated | 328 ± 32 | 52 | 393 ± 59 | 46 | 334 ± 28 | 58 |
| Without cells | 689 ± 50 | 0 | 730 ± 49 | 0 | 785 ± 28 | 0 |

PFC–Count/$2 \times 10^6$ spleen cells.

**Table 4** *Activity of suppressor factors adsorbed on anti-DV-antisera immunosorbant columns*

| $SF/SF_2$ adsorbed on columns | SF PFC | SF % Suppression | $SF_2$ PFC | $SF_2$ % Suppression |
|---|---|---|---|---|
| Normal sera | 414 ± 56 | 50 | 393 ± 49 | 52 |
| Anti-DV-antisera | 718 ± 54 | 22 | 410 ± 53 | 50 |
| Unadsorbed | 291 ± 46 | 65 | 397 ± 32 | 52 |
| Without $SF/SF_2$ | 819 ± 35 | 0 | 824 ± 84 | 0 |

PFC–Count/$2 \times 10^6$ spleen cells.

of SF was 65% which was reduced to 22% on incubation with anti-DV-antisera coupled columns. The suppressor activity of $SF_2$ was unaffected by adsorption on such column. The suppressor activity of SF and $SF_2$ adsorbed on normal mouse coupled column was 50–52%.

## DISCUSSION

The three subpopulations of the suppressor T cells induced by DV are similar to each other in many respects but differ in others (see table 5). One of the dissimilarities is that they

**Table 5** *Comparison of the three subsets of DV-induced suppressor T cells*[1,3,10,23–28]

| Features | Suppressor cells $Ts_1$ | $Ts_2$ | $Ts_3$ |
|---|---|---|---|
| Similarities | | | |
| Thy phenotype | $Thy1.2^+$ | $Thy1.2^+$ | $Thy1.2^+$ |
| I-J | Positive | Positive | Positive |
| I-A | Positive | Positive | Positive |
| Antigen specificity | Specific | Specific | Specific |
| Genetic restriction | Present | Present | Present |
| Dissimilarities | | | |
| Inducer | DV | SF | $SF_2$ |
| Induction needs MØ | ? | Yes | No |
| Ly phenotype | $Ly2^+$ | $Ly2^+$ | $Ly1^+$ |
| Cyclophosphamide | Resistant | Resistant | Sensitive |
| Soluble product | SF | $SF_2$ | Nil |
| Receptor binds# | DV-antibody | DV-antigen | DV-antibody |

# Present study; MØ–macrophages.

had receptor sites either for the DV antigen or for the antibody. It was observed that DV-specific antigen binds with and blocks the activity of $Ts_2$ and its product $SF_2$ while the anti-DV-antibody binds with and blocks the activity of $Ts_1$, its product SF and $Ts_3$. Thus $Ts_1$, SF and $Ts_3$ have antigenic determinants reflecting the conformation of antibody combining sites, that is idiotypes, while $Ts_2$ and $SF_2$ have anti-idiotypes.

The T cells require the presence of H-2 gene products or accessory cells to bind antigen but the suppressor T cells can bind antigen without them. Suppressor T cells and their factors can also bind reagents specific for $V_H$ and/or idiotypic determinants on immunoglobulin molecules[13–16]. Thus, some suppressor T cells can bind antigen via idiotypic receptors while others have anti-idiotypic receptors that can serve as an internal image of antigen. The ability of suppressor T cells to bind antigen has been shown by adherence to the antigen-coated polysterene surface from which enriched cell population can be recovered by the temperature shift[14,16–18]. However, the ability of different subpopulations of suppressor T cells to bind antigen may vary in different systems. In 4-hydroxy-3 nitrophenyl acetyl and azobenzenearsonate systems, $Ts_1$ and $Ts_3$ subsets have antigen-binding receptors while $Ts_2$ subset has anti-idiotypic receptors[5]. This is just the reverse of what has been observed in the present study of DV-model.

The developing T lymphocytes which bind antigen through their receptors become T suppressor cells while those which do not bind antigen through their receptors evolve a T-helper or cytotoxic T cell[19]. The suppressor factors produced in mice treated with GAT or GT have been shown to be associated with a small quantity of antigen or its fragment[20]. $Ts_1$ cells are induced by DV and it is likely that some DV-antigen is carried by them in the form of a complex with the receptors or have idiotypes behaving like external antigen, thus imparting antigen-specificity to their functions. The idiotypes expressed by T cells responding to a particular antigen are similar to those on immunoglobulin (Ig) molecules specific for the same antigen. This could be due to the determination of the final T-cell idiotype repertoire by idiotypes expressed by B cells to which maturing T cells are exposed[8]. Therefore, binding of anti-DV-antibodies to $Ts_1$, SF and $Ts_3$ is not surprising.

Further, SF was adsorbed by anti-DV-antibody immunosorbant columns and $SF_2$ was not. Thus SF has the properties of a soluble form of $Ts_1$ receptor and the $SF_2$ that of $Ts_2$. The suppressor factors can be divided into two classes; the first includes 'classical factors', being antigen$^-$, idiotype, anti-idiotype or allotype-specific, I-J$^+$ and acting via a complex cascade of cells. The second includes Ig-binding, isotype-specific factors produced by corresponding Fc-receptor-positive T cells. Interestingly $SF_2$ belongs to the first class while the SF having all the characteristics of the factors of first class shares the property of binding Ig with those of second class of suppressor factors. The suppressor factors which could be divided into the above two classes are induced by synthetic antigens while the ones described in the present study are induced by a virus. A similar factor which binds Ig and shares the property of the first class has been described by Daley *et al*[21] which is produced by a radiation leukaemia virus transformed T-lymphoma cell line. Thus, virus-induced factors may belong to a distinct class which share properties of the both.

An interesting feature of DV-induced suppressor pathway is the antigen specificity and genetic restriction. Further, the activity of different constituents of the suppressor pathway is blocked by binding with DV-antigen or the antibody against it. The suppression can only be mediated if the circuit from $Ts_1$ to $Ts_3$ is completed; if any component is removed or blocked it did not occur viz. removal of macrophages or depression of $SF/SF_2$ production[1,2]. In picryl chloride system the suppressor factor molecule has atleast three recognition sites, one for the antigen, second for the I-J determinants, and the third for the T acceptor cell. Appropriate binding of the two receptors (for

antigen or idiotype and MHC product) is essential for transmission of the suppressor signal[22]. This has been confirmed in the present study by abrogation of suppression by blocking the receptors of the suppressor pathway by complementary reagents. The present findings thus demonstrate the existence of a network of idiotypic-anti-idiotypic-like interactions in the DV-induced suppressor pathway.

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