

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 functions¹⁻³.

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

Jerne⁶ 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 — idiotype-anti-idiotype interactions. Thus, according to the general scheme, if Ts_1 expresses the anti-idiotype or idiotype; it can bind the antigen⁵ and immunoglobulins⁷ and may express idiotype determinants similar to the dominant idiotypes characteristic of antibodies against the inducing antigens⁸. 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 elsewhere⁹.

Preparation of Ts_1 cells and SF

Preparation of Ts_1 cells and SF has been described earlier¹⁰. In short, spleens were collected from sick moribund mice given 10^3 LD₅₀ 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 SF₂

Preparation of Ts_2 cells and SF₂ has been described earlier¹⁰. In brief, normal mouse spleen cells (1×10^7 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 SF₂.

Preparation of Ts_3 cells

SF₂ induces the normal mouse spleen T lymphocytes to produce Ts_3 cells¹⁰. Briefly, 1×10^7 normal spleen cells treated with 0.4 ml (undiluted) of SF₂ 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% CO₂. 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 LD₅₀ of DV (i.p.) were given 48 h later 5×10^6 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 Nordin¹¹ as described elsewhere¹². From each mouse spleen multiple slides were prepared. Mean value \pm 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

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

DVMB—DV-infected mouse brain tissue homogenate; PFC—Count/ 2×10^6 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 Ts₁ and Ts₃ cells was not affected by pretreatment with DV-infected brain antigen. In contrast, the suppressor activity of the Ts₂ 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 SF₂ (10⁻¹ dilution) and was incubated at 4°C for 1 hr. 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/SF₂ 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 SF₂ 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 Ts₂ cells with anti-DV-antisera had no effect on their suppressor activity which was 46–50%. On the other hand, the suppressor activity of Ts₁ and Ts₃ cells was reduced to 12–13% from 52 to 58% by treatment with anti-DV-antisera.

Effect of adsorption of SF and SF₂ 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 SF₂ 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

Dilutions of SF/SF ₂	Unadsorbed		Adsorbed with DVMB		Adsorbed with NMB	
	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 × 10⁶ spleen cells.

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

Treatment of cells	Ts ₁		Ts ₂		Ts ₃	
	PFC	% Suppression	PFC	% Suppression	PFC	% 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 × 10⁶ spleen cells.

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

SF/SF ₂ adsorbed on columns	SF		SF ₂	
	PFC	% Suppression	PFC	% 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 × 10⁶ spleen cells.

of SF was 65% which was reduced to 22% on incubation with anti-DV-antisera coupled columns. The suppressor activity of SF₂ was unaffected by adsorption on such column. The suppressor activity of SF and SF₂ 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, 21-28}

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 molecules¹³⁻¹⁶. 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 polystyrene 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 azobenzene arsonate systems, Ts_1 and Ts_3 subsets have antigen-binding receptors while Ts_2 subset has anti-idiotypic receptors⁵. 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 cell¹⁹. 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 fragment²⁰. 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 idiomere repertoire by idiotypes expressed by B cells to which maturing T cells are exposed⁸. 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⁻, idiomere, anti-idiomere or allo-type-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 at least 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 idio type and MHC product) is essential for transmission of the suppressor signal²². 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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