DENGUE VIRUS-INDUCED SUPPRESSOR PATHWAY

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ABSTRACT

Dengue virus induces a suppressor pathway in mice which involves sequential production of three generations of T suppressor cells (Ts_1, Ts_2, Ts_3). Ts_1 cells produce a soluble suppressor factor (SF) which transmits the signal via macrophages to generate Ts_2 cells which produce a soluble prostaglandin-like factor (SF_2) to recruit Ts_3 cells which suppress antigen-specific IgM antibody plaque forming cells. The properties of these supressor cells and their products have been discussed as a model in relation to pathogenesis of the disease. It is concluded that the generation of suppressor pathway is a protective phenomenon.

IMMUNE response involves a complex series of interactions between macrophage (MØ), T and B cells, initiated by antigenic stimulus. The modulators of immune response are suppressor cells which may be antigen-specific or non-specific in effector functions[1]. In recovery from infections, immune response is of paramount importance but may result in immunopathology in several of them. Therefore, regulation of immune response is crucial in infectious diseases. Synthetic antigen-induced suppressor cells and their products have been studied in depth and their suppressor pathways have been established[2]. The only infectious agent used to delineate the sequential events in suppressor pathway is the dengue virus which has been presented here as a model of chain of events in an infection.

Induction of suppressor cells: Induction of suppressor cells occurs by overloading of immune system with higher doses of antigen; antigen is presented directly to the immune cells bypassing macrophages (MØ), through formation of immune complexes and due to genetic predisposition. In dengue type 2 virus (DV) infection of mice, the virus replicates in spleen besides other organs[3–5], thus overloading with antigen; the MØ are killed or become functionally defective[6–9], presence of immune complexes have been shown in human infection[10]. Therefore, the above factors, singly or combined in various proportions provide a strong stimulus for the generation of suppressor cells. DV induces suppressor cells both by i.p. or i.c. routes and the activity lasts for 3 weeks[4, 19]. In other systems the suppressor activity wears off in 2 to 6 weeks[11–13].

Suppressor cell types: Suppressor cells are mostly T lymphocytes and are capable of inhibiting both thymus-dependent and thymus-independent immune responses[11–15]. In other systems sometimes suppressor B cells[16, 17] or suppressor MØ[18] have been noticed. The DV-induced suppressor cells of the first (Ts_1)[19], second (Ts_2)[20] and third generation (Ts_3)[21] are T lymphocytes as shown by abrogation of their activity by treatment with Thy 1.2 antibody and complement or through mediation of suppression by enriched T cell subpopulation but not by enriched B cells or MØ.

Ly phenotype: T cells have gene coded Ly antigens on their surface which are not found on any other cell. Recently a number of Ly antigens have been described[22] but still the T cells are divided into three subsets having Ly123, Lyl or Ly23 antigens. The phenotype of helper T cells is Ly1, while that of suppressor and cytotoxic T lymphocytes is Ly23 and the precursor cells have Ly123. The phenotype of DV-induced Ts_1 and Ts_2 cells is Ly23 and that of precursor of Ts_2 is Ly123[23]; on the other hand the Ts_3 cells are Ly1 and their precursors are Ly123[21]. The suppressor cells for humoral response are Ly23[24] while those for DTH against horse RBC, influenza virus and Leishmania tropica have been reported to be of Ly1 type[25–27]. Lyl cells also act as inducers of suppressor activity[28]. In DV-model Ts_1 and Ts_2 appear to be suppressor cells while the Ts_3 cells appear to induce suppression of B cell activity.

Cyclophosphamide sensitivity of suppressor cells: Cyclophosphamide (CY) has been used extensively to analyse the immune response. It has a short half-life and acts on immunologically competent and actively

dividing cells[29]. Two precursor subpopulations of T lymphocytes sensitive to CY are the helper T cells for antibody production and the suppressor T cells for DTH[30, 31]. Thus deletion of suppressor T cells by CY treatment enhanced DTH response in a number of systems[31-33]. In DV-model Ts_1 and Ts_2 cells are CY-insensitive while Ts_3 cells are CY-sensitive[34]. This further supports the conclusion that Ts_3 cells are the inducers.

Production of soluble suppressor factors: The suppressor cells may mediate their activity either through direct contact of interacting cells or through elaboration of soluble factors. In DV-model the Ts_1 cells produce a soluble suppressor factor (SF) that can be extracted from cells or is secreted in culture fluid. SF remains in supernatant at 103,500 g centrifugation and can be purified by gel electrophoresis or Sephadex column chromatography. It is dialysable, acidic and alkaline pH-sensitive, heat-labile and trypsin-resistant. It is actively synthesized by the cells as its production is inhibited by treatment with mitomycin C or cycloheximide[35, 36]. SF induces another subpopulation of T lymphocytes *in vivo* and *in vitro* to produce another soluble factor (SF_2). The SF_2 is prostaglandin-like as its production is inhibited by pretreatment with indomethacin which is an irreversible inhibitor of prostaglandin synthetase enzyme. SF_2 can be extracted from as well as secreted by Ts_2 cells[36, 37].

Neither the molecular structure of the suppressor factors nor the nature of their receptors on cells is known so far. Active efforts are being made in this direction. Recently Taniguchi *et al*[38] have reported reconstruction of antigen-specific suppressor activity with translation products of mRNA isolated from cloned T suppressor cells and injected in Xenopus oocytes. Similar findings have been reported by Wieder *et al*[39] by injecting mRNA in rabbit reticulocytes.

Antigen-specificity of suppression: The production of a suppressor factor may be specifically antigen-induced but its effector functions may be antigen-specific or non-specific. Suppressor T cell factors produced in L-glutamic acid[60]-L-alanine[30]-L-tyrosine[10] (GAT), L-glutamic acid[50]-L-tyrosine[50] (GT) inoculated mice are antigen-specific in effector function also[40]. But those against keyhole limpet haemocyanin (KLH) are produced in the presence of specific antigen and produce a non-specific suppressor effect[41]. Inoculation of mice with SF, SF_2 or SF-adsorbed MØ in DV-primed mice or spleen cell cultures lead to suppression of DV-specific IgM-PFC but has no effect on Coxsackie B_4 virus, Japanese encephalitis virus or sheep RBC-specific IgM-PFC. Even in a Mackaness type of experiment where mice were primed simultaneously with DV and an unrelated antigen the suppression of PFC against heterologous antigen did not occur[20, 35, 42]. Thus, DV-induced suppression remains antigen-specific throughout. How even SF_2 retains antigen-specificity is not well understood; it may be because the signal for release comes from antigen committed cells[43]. The suppressor factor produced in GAT or GT treated mice is associated with a small quantity of antigen or their fragments and the second generation of T lymphocytes (Ts_2) induced by it are absolutely specific to antigen which stimulated initial suppressor cells[41]. In our model the signal for production of Ts_2 and SF_2 comes from specific DV antigen-primed cells, which probably imparts the antigen specificity.

Genetic restriction on suppressor activity: A requirement of I-J subregion identity between KLH induced suppressor factor and the KLH-primed T cells for mediation of suppression has been described by Tada *et al*[44]. Activity of DNP suppressor factor on DNP immune cells has been shown to be H-2D restricted[45]. Similarly, genetic identity of interacting cells is required for mediation of DV-specific suppression of PFC in DV- primed spleen cells. SF was neither adsorbed on MØ nor suppressed PFC in DV-primed spleen cells obtained from guinea pigs, albino rats, monkeys or mice of Parker strain. The suppression occurred only in syngeneic spleen cells of Swiss albino mice[46]. We have not yet studied the precise effect of MHC restriction on DV-induced suppressor pathway, but the absence of suppressor activity even in cells of Parker-strain mice is a strong indicator of such a possibility. In contrast, the necessity of genetic identity does not exist in some models. A number of experiments carried out with GAT and GT suppressor factor show suppressor activity across the H-2 barrier also thus showing absence of a need for genetic matching.

Subpopulations of T cells in suppressor pathways: Study of suppressor cells in various infections including those with viruses has been restricted to the demonstration of their presence only but no attempts have been made to investigate their mechanism of action. On the other hand, synthetic antigen-induced suppressor pathways have been established which have up to three generations of T cells[40]. The main parameters used in these studies to characterize the suppressor cells have been the surface phenotype, H-2 (I-J) and V_H restriction, antigen-specificity and CY-sensitivity of the cells. The

only infectious agent used to delineate such a sequential suppressor pathway has been the DV in which also three generations of the T cells have been established so far (figure 1). DV-induced Ts_1 cells are Thy 1.2^+, Ly23, species-restricted, antigen-specific and CY-insensitive[19, 20, 23, 34, 37, 46]. The Ts_2 cells are Thy 1.2^+, Ly23, species-restricted, antigen-specific and CY-insensitive[20, 21, 23, 34]. The third generation of DV-induced T cells (Ts_3) are Thy 1.2^+, Lyl, antigen specific, species-restricted and CY-sensitive[21,34]. One of the criteria suggested by Germain and Benacerraf[40] in their unifying scheme of suppressor pathways is the sensitivity of the suppressor cells to CY. The findings of CY-insensitivity of DV-induced Ts_1 and Ts_2 cells do not disqualify them from being classed as a suppressor cell because Shand and Liew[31], using sheep erythrocyte as antigen, have shown that the suppressor T cells for DTH and the helper T cells for the humoral response are CY-sensitive. In DV model the indicator of suppression are IgM-PFC (humoral response); therefore in the light of the findings of Shand and Liew[31] and on the basis of Ly1 surface phenotype Ts_3 cells appear to be an inducer T cell.

Induction of DTH to DV in CY-treated mice has been reported by Pang *et al*[33]. But all our attempts to induce cell-mediated immune response including DTH against DV in inbred Swiss albino mice have failed and even pretreatment of such mice with CY did not elicit any DTH[4] (and unpublished data). Could this be due to simultaneous induction of CY-insensitive suppressor cells for DTH against DV? Simultaneous induction of suppressor cells for antibody production and DTH have been reported in a number of studies including those on sheep erythrocytes[47] and Japanese encephalitis virus[12, 13, 48].

The third generation of T cells in KLH, dinitrophenyl (DNP) and azobenzenarsonate (ABA) induced suppressor pathways are the suppressor (Ts_3) cells[40] while in the present model Ts_3 cell is an inducer cell having a surface phenotype Ly1. In various immune responses Ly1 T cells act as inducer or initiator cell and among them cells of I-J^+ subset induce suppressor activity while cells of I-J^- subset induce B cell activity[28]. Further, in other models Ts_3 cells are generated in antigen-primed cells while in DV-model Ts_3 cells are induced in unstimulated normal mouse spleen cell cultures[21]. The suppression of PFC can be brought about in two ways: (i) either by direct suppression of B cells by the suppressor cells or their products, or (ii) by preventing the help through suppression of helper cells. With the data available in DV-model Ts_3 cells appear to induce suppression of B cell activity. Studies, including I-J phenotyping, are required to further characterize these cells and their mode of action.

Gershon *et al*[49] have described a contrasuppressor cell which expresses the phenotype Ly123, I-J^+, Qa-1^+ and regulates the activity of the suppressor T cells. On the other hand it has been proposed recently that T suppressor may convert to T helper cells and the reverse may also happen thus regulating the activity of these cells[50, 51].

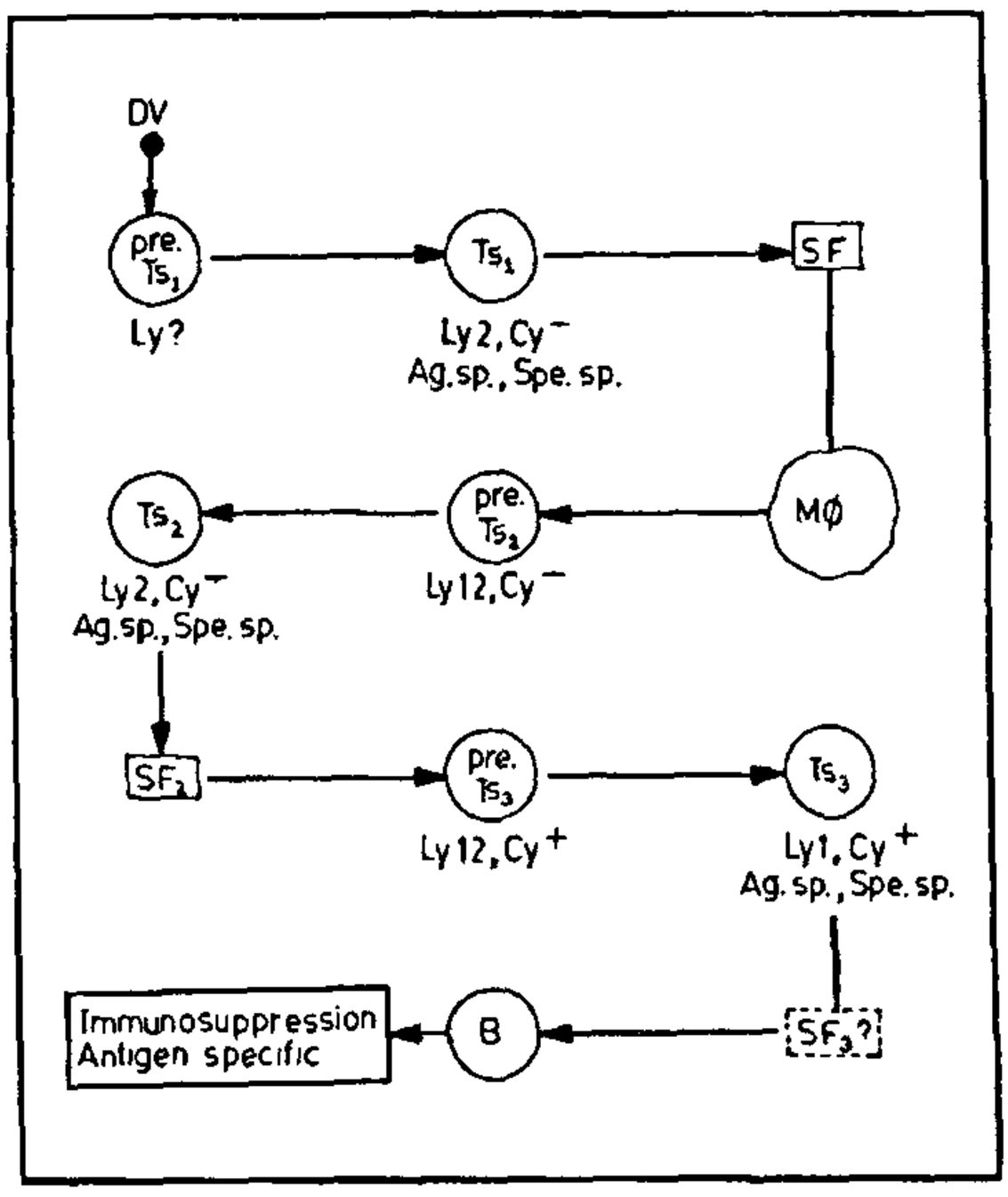

Figure 1. The suppressor pathway induced by dengue virus (DV). Shows suppressor T cells of first (Ts_1), second (Ts_2) and third (Ts_3) generation; their precursors (Pre) and soluble products (SF and SF_2) and the macrophage (MØ). Also indicated are the surface phenotype (Ly): cyclophosphamide sensitivity (CY$^+$) or resistance (Cy$^-$); antigenic specificity (Ag. sp.); species specificity (Spe. sp.); and B lymphocyte (B).

Transmission of suppressor signal: Sequential induction of the three generations of T lymphocytes in DV-model raised the question of transmission of signal from one generation of T cells to another. During studies on transmission of signal from Ts_1 to Ts_2 cells it was observed that SF is adsorbed on the surface of MØ. Live MØ adsorbed SF, retrieved it from that adsorbed on heat-killed MØ and presented it to the target cells.

Heat-killed MØ adsorbed SF to the same extent as live ones, but could not present it to the target cells by themselves. SF was adsorbed on syngeneic MØ but not on those from allogeneic animals[46]. These findings were confirmed in *in vivo* experiments using MØ-depleted mice by silica treatment[42]. It is, therefore, concluded that *live MØ-like cells are essential for* transmission of suppressor signal from Ts_1 to Ts_2 cells. The crucial role of MØ in induction of suppressor cells and *transmission of suppressor signal has been shown* by Pierres and Germain[52] and Howie and Feldmann[53]. The mechanism of transmission of suppressor signal between Ts_2 and Ts_3 cells is not yet well understood. All attempts to find a cell which may attach SF_2 have failed so far, it, therefore, appears that SF_2 acts directly on precursor cells to recruit Ts_3 cells[21].

Transmission of signals from one cell to another can be mediated either through secretion of a soluble mediator or by close contact of the plasma membranes of the interacting cells. SF-adsorbed MØ neither release SF nor any other soluble product which may mediate suppression of DV-specific IgM-PFC. This conclusion has been drawn from the two experimental data: SF-adsorbed MØ fail to transmit suppressor signal when separated from spleen cell cultures through cell impermeable membranes; and absence of suppressor activity in the culture fluid of SF-adsorbed MØ[54].

The adsorbed SF remains only at the surface of the MØ and can be retrieved from them completely. It is not known how SF remains tightly bound to the surface of MØ but we know that the SF is adsorbed only on syngeneic MØ and suppressed DV-specific PFC *in syngeneic spleen cells*[46]. Thus, binding of SF through MHC products can be a strong possibility and remains to be further investigated as discussed elsewhere[54].

The second mechanism through which the signal can be transmitted is by close contact of plasma membranes of the MØ and T lymphocytes. This can be the mechanism of the transmission of the suppressor signal in DV-model as the suppressor signal is not transmitted when the cells are separated by a cell impermeable membrane[54]. Rosenthal *et al*[55] have described three types of physical interactions between MØ and lymphocytes for transmission of antigen-specific signal. Active metabolism, divalent cations and a trypsin sensitive site are required on MØ in the first type. The binding in this reaction can be with T or B cells and is reversible[56]. The second type of binding is antigen-specific, not reversible easily, and is dependent upon the presence of antigen and a sharing of MHC linked gene products and MØ and lymphocytes[57]. The third type of binding is non-antigen-specific, easily reversible and occurs when lymphocytes cluster about the antigen-specific central lymphocytes[58]. It appears that the binding of macrophage-lymphocyte in our model is of the second type of Rosenthal *et al*[55].

Biological significance of suppressor system: Suppressor cells are the regulators of immune response and are thus of prime importance in preventing immunopathological consequences of over-reaction of the immune system. Dengue virus causes a benign febrile illness (DF) and also a severe type of illness, the dengue haemorrhagic fever (DHF) and the dengue shock syndrome (DSS). DHF/DSS occur during secondary infection with the virus, predominantly in certain areas of the world. The pathogenesis of the illnesses is not fully understood but immunopathological processes appear to play a significant role. Initially it was thought to be mediated by antigen-antibody immune complex[59]. Recently the role of enhancing antibody has been suggested which augments the uptake of the virus by the macrophage/monocyte through Fc-receptors, thus enhancing the replication of DV in them. It has been proposed that during 'immune elimination', probably by T lymphocytes, the virus-infected MØ release pharmacologically active substances which produce symptoms[60, 61]. Recently, Pang[62] has envisaged a role of T lymphocytes mediating DTH in such responses.

The role of suppressor phenomenon in controlling such chain of reactions can be crucial. DV-induced suppressor pathway suppresses antigen-specific antibody production. Thus increased replication of the virus mediated by the enhancing antibody and the immunopathology mediated by the immune-complex is prevented. Further, in certain strains of mice DTH can be induced[33] while in others it is not possible[4, 63] (unpublished data). In 'non-responder' strains where immune response does not develop against an antigen the role of suppressor cells has been suggested[2]. In primed mice DV-induced suppressor cells last for 3 weeks[4] and on adoptive transfer the suppressor activity of Ts_1 cells persist for 3 weeks in recipient mice and that of SF, Ts_2 and SF_2 from 1–2 weeks[64]. Suppressor T cell activity, regulating B and T cell response in dengue type 3 virus-infected mice has been shown[65]. Induction of suppressor cells in DV may suppress CMI and thus protect the body against T cell-mediated damage. Recently Liew and Russell[66] have presented direct evidence of the beneficial effect of specific suppressor T cells for DTH against potentially

lethal influenza A virus infection in mice. Thus, induction of suppressor pathway in DV-model appears to be a protective phenomenon.

The data from mouse model cannot be extrapolated to man but they strongly indicate the possibility of the existence of such a mechanism in man as well, which determines the outcome of infection. There is a need to investigate human cases of dengue infection on similar lines. The biological significance of a multistep pathway is in self-regulation and amplification of the signal. Product of one T cell can activate more than hundred cells thus amplifying the signal. Immunosuppression is frequently observed in a number of viral infections but the precise mechanism of its genesis is not known[67]. The unique sequential mechanism of immunosuppression in DV, as far as we are aware, has been described for the first time in an infectious disease and indicates a need to investigate other models to see if this is the basic mechanism inolving other systems as well.

In conclusion, it is obvious that a lot of progress has been made in delineation of the suppressor pathway in a virus infection but much still needs to be done. The future will see resolution of the chemical nature of the suppressor factors, their mechanism of transfer of signal, their receptors on the cells and the mechanism of effector functions. There is need to understand the precise mechanism of the suppression, feedback regulation of each step and the maintenance of the antigen-specificity till final stage of the action. Information on these aspects will help in a better understanding of immunoregulation and the pathogenesis of infectious diseases.

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NEWS

X-RAY TUBE

Phillips has introduced a new side-window x-ray tube for sequential spectrometry. The model PW 2180 features a scandium anode which, the company claims, enhances excitation of light elements up to calcium (atomic number 20), whilst maintaining good results for heavy element analysis. The anode produces low level radiation that is sufficiently energetic to give good transmission through the beryllium tube window. For heavy elements the PW 2180 allows operation up to 100 KV. Side window tubes do not require deionised cooling water and the end cap geometry permits closer coupling to the spectrometer optics. (*Chemistry in Britain*, Vol. 20, No. 3, p. 201).