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Differences in Stimulation of CD45+ Cells from Mouse Spleen Population Enriched in Dendritic Cells Depending on the Virulence of Herpes Simplex Virus Type 1 Strains

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Abstract

The interaction of CD28 with one of the B7 molecules (CD80 and CD86) on professional antigen-presenting cells (APC) is generally considered to be the most important co-stimulatory signal for T cell activation. Several lines of evidence suggest that dendritic cells (DC), the most potent antigen presenting cells known, play a role in the immunological control of herpes simplex virus (HSV) infections. The fact that CD86 is strongly up-regulated together with other co-stimulatory molecules during DC maturation suggests that it plays an important role in induction of immune response. To determine the effect of virulence on up-regulation of CD86, we stimulated population of spleen cells enriched in dendritic cells by HSV-1 strains characterised by different pathogenicity. We analysed cells, which express CD45 molecule. HSV-1 ts, earlier described as less virulent for mice, stimulated an increased expression of co-stimulatory molecule CD86 than wild strain did.

Key words: CD45+ cells, immune stimulation, HSV-1

Introduction

Herpes simplex virus type 1 (HSV-1) belongs to the Herpesviridae, a large and diverse family of vertebrate pathogens including several human pathogens such as HSV-1 and HSV-2 (Roizman, 1990). Members of the Herpesviridae characteristically have a large double-stranded DNA genome, and virions consist of an icosahedral nucleocapsid surrounded by a lipid layer envelope. After the initial acute infection of the host, HSV-1 establishes a persistent infection. Such persistence is established when host immune response fails to eliminate the virus, which is produced during the acute stage of infection. A protective immune response to HSV is critical in resolving highly lytic primary HSV infection, since failure to do so can result in encephalitis and even death, a condition observed in new-borns and immunocompromised hosts (Burchett *et al*., 1992).

The antigen-specific response to a viral pathogen is initiated when a T cell recognises a viral peptide presented in the context of major histocompatibility complex (MHC) on antigen-presenting cells (APC). This primary signal results in activation of T cell, the extent of activation being a function of both the affinity and duration of this interaction (Iezzi *et al*., 1998; Kundig *et al*., 1996). Low affinity or brief primary signals can result in insufficient T-cell activation unless augmented by secondary interactions called co-stimulatory signals (CS). The best characterised co-stimulatory signals determined to be important in the initiation of the immune response are the CD28/CD80-86 and CD40/CD154 receptor-ligand interactions (Bluestone, 1995; Grewal *et al*., 1997; Noelle, 1996). In addition to their essential role in the development of the antigen-specific humoral response, they appear to facilitate T-cell activation in response to low-affinity or low-abundance antigens by lowering the threshold required for activation and by promoting survival of activated T cells (Bachmann *et al*., 1996; Kundig *et al*., 1996; Sperling *et al*., 1996, Viola and Lanzavecchia, 1996)

We stimulated mouse spleen population enriched in DC with HSV-1 strains characterised by different pathogenicity to determine the affect of virulence on expression of the co-stimulatory molecule – CD86. We used temperature sensitive mutant 28°C (HSV-1 ts) and temperature resistant 39°C (HSV-1 tr) isolated by

Litwińska *et al*., 1991, from McIntyre strain. It was defined earlier, that HSV-1 ts is less virulent for mice and establishes latent infections rarely and HSV-1 tr is more virulent for mice than native strain is (Litwińska *et al*., 1996; Litwińska *et al*., 2001).

Experimental

Materials and Methods

Viruses. HSV-1 strain McIntyre, obtained from Institute of Hygiene in Freiburg, temperature sensitive mutant 28°C (HSV-1 ts), and temperature resistant 39°C (HSV-1 tr) were grown and titered on CV1 cells.

Enrichment spleen population in DC. Spleens obtained from BALB mice were digested using collagenase D (Sigma) and next were spun down for 10 min at 280×g at 4°C. The cell pellet was resuspended in dense bovine serum albumin (BSA) (Sigma) ~1 ml per spleen. Each 5 ml of cell suspension were overlaid with 1,5 ml of RPMI-1640 medium (GIBCO) and centrifuged for 15 min at 9000×g at 4°C. The cells from the interface region were collected and washed with RPMI-1640 by centrifugation. Then, next measures were taken, the pellet was resuspended in RPMI-1640 + 5% FCS (foetal bovine serum) (Sigma) to 10^7 cells/ml, plated in volume of 4 ml on a 60 mm dish and incubated for 90 min. The non-adherent cells were removed by gentle washing with RPMI-1640. Plastic-adherent cells from this fraction were primarily dendritic (~80%) but other cell types such as macrophages and B cells were present. The medium was replaced by RPMI-1640 + 5% FCS and the cells were incubated for 20 h. Then cells were harvested, centrifuged and the pellet was resuspended in fresh RPMI-1640. 1 ml cell suspension was overlaid on 5 ml dense BSA and centrifuged with the same parameters. The interface cells were collected.

Antigen stimulation of population enriched in DC. The obtained cells were seeded on 24-well plates, 5×10^5 cells per well. 1×10^4 pfu of HSV-1 or HSV-1 ts or HSV-1 tr were added to well (moi 0.5) and culture was incubated for 24 h at 37°C in 5% CO_2.

Flow cytometric analysis. To detect surface molecule expression, cells were originally washed with washing buffer (HBSS containing 3% FCS, 1 mM EDTA, and 10 mM HEPES). All subsequent steps were conducted in this buffer on ice. After blocking Fc receptor by using 1 μg of anti-mouse CD16/CD32 Mab (BD Pharmingen) per 5×10^5 cells for 10 min, cells were stained with antibodies, (CD86, CD45 and rat IgG2a – BD Pharmingen) directly conjugated to fluorescein isothiocyanate (FITC) or phycoerythrium (PE), for 30 min. Cells were fixed by CellFIX (Becton Dickinson) and then analysed by two-colour flow cytometry. All samples were acquired using a FACSCalibur flow cytometrer (Becton Dickinson) and were analysed using CellQuest Software (Becton Dickinson).

Statistical analysis. Statistical analyses were performed with Statgraphic Plus for Windows (Manugistics).

Results

To investigate the effect of virulence of different strains of HSV-1 on the expression of co-stimulatory molecule CD86 by CD45+ cells from mouse spleen population enriched in dendritic cells, culture was examined by two-colour flow cytometry. The cells were infected with HSV-1 ts, or HSV-1, or HSV-1 tr at moi 0.5 and analysed 24 h after infection. Controls included non-infected cells and isotype-matched antibody controls.

After HSV-1 ts infection approximately 15% of cells showed positive staining for CD86 and CD45 (Fig. 1). There was significant difference between cells infected HSV-1 ts and control cells (non-infected), and cells infected with HSV-1 and HSV-1 tr. This effect wasn't observed when cell population was inoculated with HSV-1 or HSV-1 tr. After HSV-1 and HSV-1 tr infection approximately 11% of cells showed positive staining for CD86 and CD45, and approximately 12% of control cells were stained positive for CD86 and CD45.

This data shows that HSV-1 ts infection induces CD86 up-regulation on CD45+ cells.

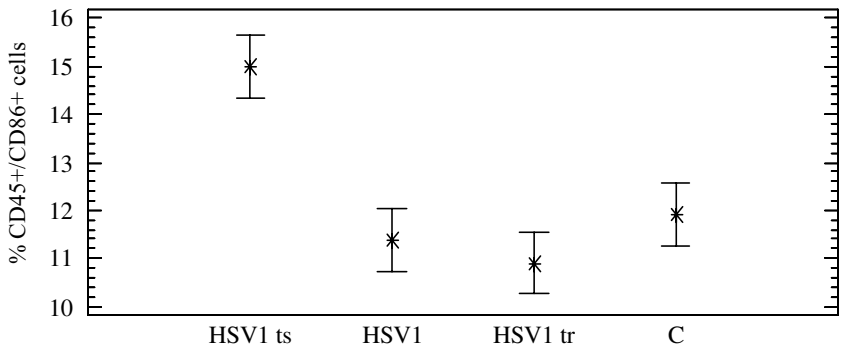

Fig. 1. The phenotype of mouse spleen population enriched in dendritic cells after 24 h incubation with HSV-1 ts or HSV-1 or HSV-1 tr

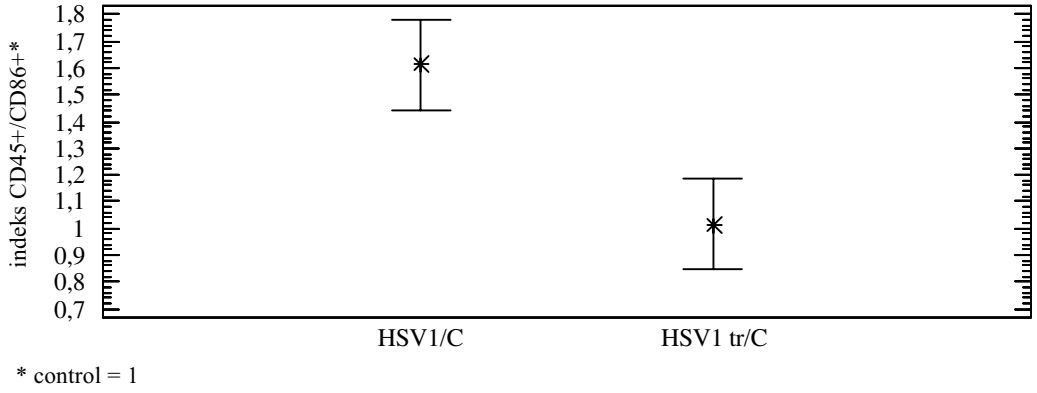

Fig. 2. Index CD45/CD86 in mouse spleen population enriched in dendritic cells after 24 h incubation with HSV-1 – 2 log or HSV-1 tr

We described earlier that population of HSV-1 ts has a lot of defective particles (Rechnio and Litwińska, 2002). Now, our aim was to find out if CD86 up-regulation was connected, or not with presence of defective virus particles. Therefore, we performed experiment in which we used the same strain of native HSV-1 as previously, yet its titer was 2 log lower (HSV-1 – 2 log). As the result, in the same volume of virus the number of infectious virus particles decreased, however there were a lot of non-active (defective) virus particles. The results were compared to results obtained from cells infected with HSV-1 tr mutant and uninfected cells (control). Our results were presented as an index of CD 45+/CD86+ to control cells (control = 1) (Fig 2). There was significant difference between cells infected with HSV-1 –2 log and HSV-1 tr.

We concluded, that CD86 up-regulation on CD45+ cells was connected with the presence of defective virus particles.

Discussion

In this study, we have shown that the exposure of mouse spleen population enriched in dendritic cells to HSV-1 ts leads to up-regulation of CD86 co-stimulatory molecule. HSV-1 ts was described earlier as less virulent for mice but manifesting high immunogenic potency (Litwińska *et al*., 2001). Moreover, HSV-1 ts showed a significantly lower possibility to cause the latent infection but the state of immunosuppression increased the frequency of latent HSV-1 ts infection (Litwińska *et al*., 1996; Litwińska *et al*., 2001). The suspension of infectious and non-infectious HSV-1 ts induced PBMC to hyperproduction of IFN alpha and we proved that there was no connection with virus infectivity, however there was one with the presence of defective particles (Rechnio and Litwińska, 2002).

Previous studies indicate IPC (IFN-alpha-producing cells) culture infected with HSV increased the expression of co-stimulatory molecules CD80 and CD86 (Kadowaki *et al*., 2000). But the findings by Salio *et al*., 1999, indicate that DC infected with HSV-1 are defective in up-regulation of co-stimulatory molecules, CD86 as well. It turned out that it was dependent on viral replication. The co-stimulatory molecules were up-regulated in response to stimulation with UV-inactivated HSV-1 (Mikloska *et al*., 2001). We suggest that the presence of plenty of defective particles in the population of HSV-1 ts may cause the same affect.

Moreover, production of IFN type I is important to promote maturation of monocytes (Hayward *et al*., 1993) and DC (Luft *et al*., 1998). Therefore, hyper-production of IFN alpha in response to infection of HSV-1 ts may help to explain up-regulation of CD86 in culture infected with HSV-1 ts.

Studying the up-regulation of CD86 we found out that it is connected with decreasing of the pathogenicity of HSV-1 ts for mice and hyper-production of IFN alpha in response to infection of HSV-1 ts. We suggest that the presence of IFN alpha essentially influences on the control of acute HSV-1 infection, also at the level of the APC.

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