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Research Article

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Cytomegalovirus Immediate Early Genes Prevent the Inhibitory Effect of Cyclosporin A on Interleukin 2 Gene Transcription

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Abstract

The use of cyclosporin A (CsA) as an immunosuppressive agent has markedly improved the clinical outcome in solid organ transplantation. However, posttransplantation infection remains a significant problem and may contribute to subsequent organ rejection. In this study the effect of cytomegalovirus (CMV) immediate early (IE) gene products on interleukin 2 (IL-2) gene transcription in the absence and presence of CsA was investigated using a transient transfection system. Jurkat T cells were transfected with plasmids expressing the CMV IE gene products or with a control plasmid. The presence of the CMV IE2 gene product abolished the inhibitory effect of CsA on IL-2 promoter activation and gene transcription. This effect was noted regardless of the time of CsA addition relative to the time of stimulation and was independent of CsA concentration. CsA had no effect on the CMV or the IL-2 receptor promoters. These studies suggest that the CMV IE gene products may play a role in graft rejection after solid organ transplantation. (*J. Clin. Invest.* 1992. 90:2136–2140.) Key words: immunosuppression • rejection • transfection • transplantation

Introduction

Cyclosporin A (CsA)[1] has markedly improved the management of graft rejection and patient survival in patients receiving allogeneic organ transplants (1). CsA is a fungal product which affects T lymphocyte proliferation, primarily by interfering with the production of interleukin 2 (IL-2) at the level of gene transcription (2–4). Some patients reject their transplanted organs in spite of receiving CsA. It is not known if these episodes of rejection in some patients correlate with loss of the inhibitory activity of CsA on IL-2 gene transcription.

One group of transplant patients who appear to have a higher incidence of complications are those with cytomegalovirus (CMV) infection (5, 6). Many transplant patients develop CMV infection during the first 1–4 mo after transplantation (7, 8). Active infection results in a sequential expression of the CMV immediate early (IE), early, and late genes (9). The CMV IE genes code for proteins which act in *trans* to regulate their own production (10–14) as well as the expression of the early and late genes (11, 13, 15, 16). We, and others, have shown that the CMV IE gene products can also upregulate a number of cytokine genes, including the IL-2 gene (17, 18). Therefore, it is possible that CMV infection can stimulate IL-2 gene transcription and, consequently, abrogate the effects of CsA.

In this study we asked whether the presence of the CMV IE gene products could overcome the inhibitory effect of CsA on IL-2 gene transcription. We cotransfected plasmid constructs containing the CMV IE genes along with a plasmid containing a reporter gene (chloramphenicol acetyltransferase, CAT) and the IL-2 promoter (IL-2CAT) into a T cell leukemia line. Our studies show that the CMV IE2 gene product can block the inhibitory effect of CsA on IL-2 gene transcription.

Methods

Cell culture. Jurkat cells, a T cell line, were grown in suspension in Rosewell Park Memorial Institute (RPMI-1640, University of Iowa Cancer Center, Iowa City, IA) medium supplemented with 10% fetal bovine serum (FBS, Sigma Chemical Co., St. Louis, MO), 2 mM glutamine and 80 μg/ml gentamicin at 37°C in an atmosphere of 5% CO_2. Cells isolated from these cultures were transfected during the log phase of their growth.

Plasmid constructs. Constructions of all CMV IE plasmids have been described previously and shown to express the bona fide viral gene products (11, 12, 14, 19). See Fig. 1 for a schematic diagram of these plasmids and Malone et al. (11) for details. The IL-2CAT plasmid used in these studies was obtained from G. Crabtree (Stanford University) and was constructed as previously described (20). It contains a 632-bp RsaI restriction fragment inserted into the cloning vector pJYMOCAT. IL-2RCAT (-317pTACCAT) was obtained from W. C. Greene (The Gladstone Institute, San Francisco) and was constructed as previously described (21). It contains a 580-bp fragment inserted into the cloning vector pJYMOCAT. pCAT760 contains the promoter-regulatory region of the human CMV IE region upstream from the CAT gene and has been described previously (14). Fig. 1 schematically displays these plasmids along with the 5′ and 3′ limits of each promoter.

Transfection. Transfections were performed using the DEAE dextran method as described previously (18, 22). Briefly, the cells were washed and resuspended at a concentration of 1×10^6 cells/ml in 10 ml of serum free medium containing DEAE dextran (250 μg/ml) for 1

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1. *Abbreviations used in this paper:* CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; CsA, cyclosporin A; IE, immediate early (gene).

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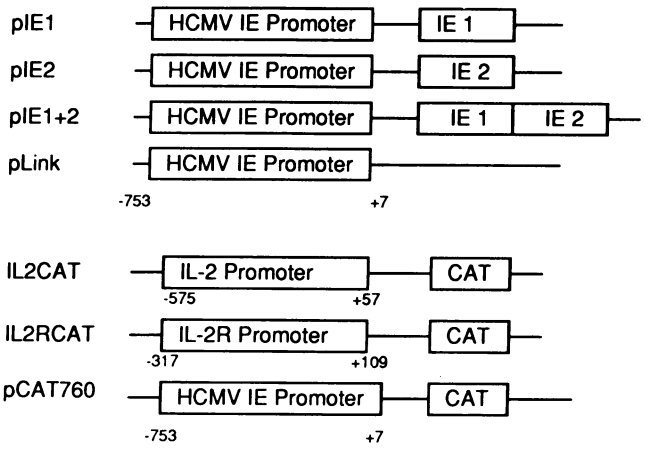

Figure 1. Schematic representation of the plasmid constructs used in this study.

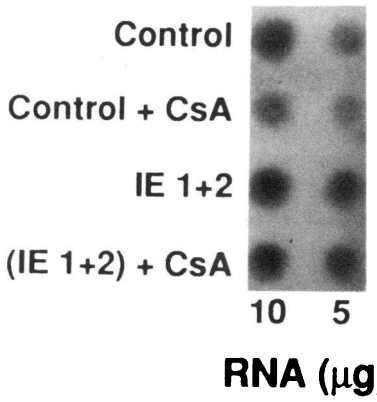

Figure 2. Effect of CsA on endogenous IL-2 gene transcription. Jurkat cells were transfected with control (pLink) or pIE1+2. 24 h after transfection, CsA (1 μg/ml) was added 30 min before stimulation with PMA and PHA. Cells were harvested after an additional 6 h in culture and whole cell RNA was extracted as described in methods. CMV plasmids and CsA treatment in on the ordinate. Amount of RNA loaded is on the abscissa.

h. The cells were then washed and resuspended in medium containing 10% FBS. Stimulation was carried out by adding phytohemagglutinin (PHA, 1 μg/ml, Sigma Chemical Co.) and phorbol 12-myristate 13-acetate (PMA, 50 ng/ml, Sigma Chemical Co.) 24 h after transfection. Cyclosporin A (CsA, 1 μg/ml, Sandoz Inc., E. Hanover, NJ) was added either 1 h before or 1 h after stimulation. The cells were harvested after an additional 24 h in culture.

CAT assay. CAT assays were performed by the method of Gorman et al. (23). Ascending thin-layer chromatography (TLC) was used to separate the acetylated derivatives of chloramphenicol using a chloroform:methanol solvent system (95:5). Autoradiograms were developed after overnight exposure. CAT activity was quantitated using a TLC scanner (Radiomatic Instruments, Tampa, FL).

Dot blot analysis. Total cellular RNA was isolated by using the guanidine isothiocyanate method (24). Dot blots were prepared by applying serial, two-fold, dilutions of whole cell RNA to a nylon membrane (Gene Screen Plus, New England Nuclear, Wilmington, DE) such that the first sample of a series contained 10 μg of RNA. Dot blots were quantitated using laser densitometry. Hybridization of ^{32}P-labeled probes was performed at 42°C. IL-2 probe (obtained from G. Crabtree) consisted of a restriction fragment length of cDNA labeled in vitro by nick translation (Bethesda Research Laboratories, Bethesda, MD). The IL-2 probe specifically recognizes a 1.0-kb mRNA species, as described in the literature (25).

Results

Effect of CsA on endogenous IL-2 RNA production. We first assessed the effects of CsA on endogenous steady-state IL-2 RNA levels in the absence and presence of the CMV IE gene products. Whole cell RNA was obtained from cells transfected with pLink or pIE1+2. Cells were treated with CsA, 30 min before stimulation. Cells were harvested 8 h later, and IL-2 RNA was quantitated using dot blot analysis. In the presence of the control plasmid and CsA there is a twofold decrease in the amount of IL-2 RNA compared with controls; in the presence of the viral gene products, CsA did not decrease the amount of IL-2 RNA (Fig. 2).

Effect of CsA on expression of IL-2CAT in the presence or absence of the human CMV IE gene products. To determine the effect of cyclosporin A on expression of IL-2CAT in the presence or absence of the CMV IE gene products, Jurkat cells were cotransfected with IL-2CAT and one of the CMV IE expression plasmids (i.e., pLink, pIE1, pIE2, or pIE1+2) For each type of cotransfection, cells were stimulated with PMA and PHA. In some experiments CsA, at a concentration of 1 μg/ml was added either 1 h before or 1 h after stimulation. Control transfections were stimulated but not treated with CsA. CsA completely inhibited IL-2CAT expression in control cells (transfected with pLink). The presence of the CMV IE1 gene product did not alter the inhibitory effects of CsA on IL-2CAT expression. The effects of CsA were seen independent of whether the cells were treated 1 h before or 1 h after stimulation with PHA and PMA (Fig. 3). As previously reported (18), the CMV IE2 gene product increased expression of IL-2CAT eightfold, compared to controls. The CMV IE2 gene product also interfered with the inhibitory effects of CsA on IL-2 gene

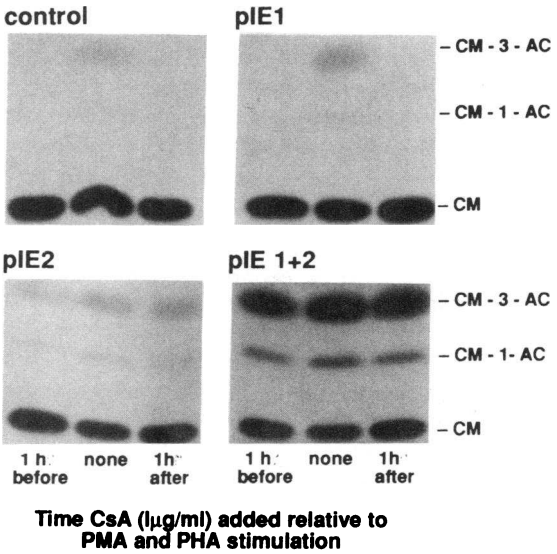

Figure 3. Effect of CsA on IL-2 gene expression in the absence and presence of the CMV IE gene products. Jurkat cells were transfected using the DEAE dextran method (18, 22) with IL-2CAT (1 μg/ml) and 1 μg/ml of either control (pLink), pIE1, pIE2, or pIE1+2. The cells were either treated with CsA (1 μg/ml) 1 h before or 1 h after PMA (50 ng/ml) and PHA (1 μg/ml) stimulation, or simply stimulated for 24 h. CAT assays were performed as described in the methods. The CMV IE plasmids are indicated on the abscissa. IL-2CAT activity is expressed on the ordinate as conversion of chloramphenicol to its acetylated derivatives.

expression. This effect was independent of the time of addition of CsA relative to the time of stimulation. The presence of both of the CMV IE gene products (IE1+2) also resulted in a synergistic increase in IL-2CAT activity (22-fold) in the absence of CsA. The presence of both of the viral gene products also completely inhibited the effects of CsA on IL-2 gene expression. It is of interest that IL-2 CAT activity was much higher (20-fold) in the presence of both viral gene products and CsA compared to control cells not exposed to CsA.

To determine whether increasing amounts of CsA could overcome the effect of the viral gene products on the IL-2 promoter, Jurkat cells were cotransfected with the IL-2CAT and either the CMV control (pLink) plasmid or pIE1+2. All cells were stimulated for 24 h with PMA and PHA. CsA, at concentrations ranging from 0.1 to 2.5 μg/ml, was added 1 h after stimulation. The presence of the viral gene products completely interfered with the inhibitory effects of CsA at all concentrations (Fig. 4). In contrast, the presence of the control plasmid had no effect. Concentrations of CsA as high as 100 μg/ml were unable to overcome the effects of the viral gene products on IL-2 gene transcription (data not shown).

To verify that CsA had no effect on the CMV promoter, Jurkat cells were cotransfected with the CMV expression plasmid pCAT760 and one of the CMV IE plasmids (pLink, pIE1, pIE2, or pIE1+2). The cells were stimulated with PMA and PHA and treated with CsA, as described above. The IE1 gene product is known to up-regulate its own promoter whereas the IE2 gene product downregulates the CMV IE promoter (10, 11). The presence of both IE gene products has an intermediate effect. The presence of CsA had neither an enhancing nor an inhibitory effect on the interaction between the IE gene products and the CMV IE promoter (Fig. 5). As an additional control, we evaluated if CsA, using the same culture conditions and CMV plasmids, altered expression of the IL-2 receptor gene, using an IL-2RCAT plasmid. As we previously reported,

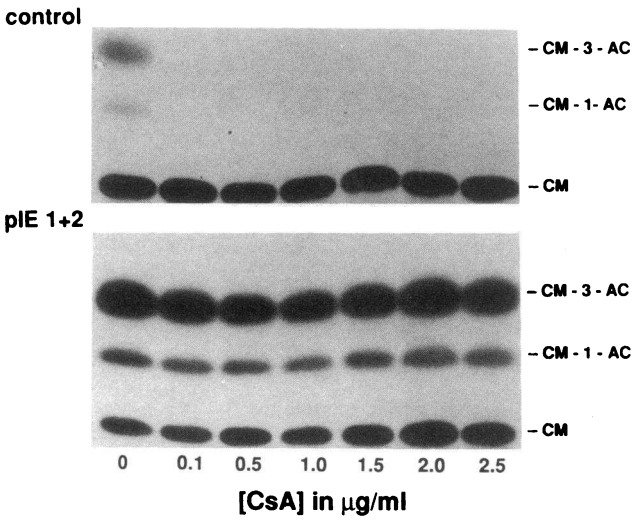

Figure 4. Dose–response curve demonstrating the effects of increasing CsA on IL-2CAT expression. Jurkat cells were transfected with the IL-2CAT and either the control plasmid (pLink) or pIE1+2. CsA was added 1 h after stimulation with PMA and PHA at the concentrations noted on the abscissa. CAT assays were performed after an additional 24 h in culture.

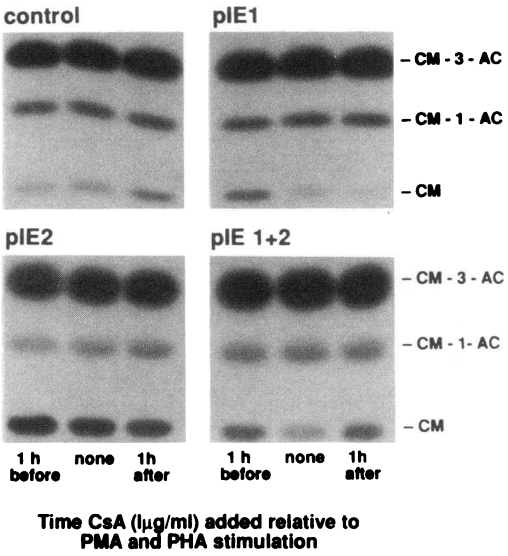

Figure 5. Effect of CsA on the CMV promoter. Jurkat cells were cotransfected with pCAT760 (1 μg/ml) and 1 μg/ml of each of the CMV IE plasmids. CAT assays were performed after CsA treatment and stimulation as described in Fig. 2. Each panel is labeled with the CMV plasmid that was used. CsA treatment is on the abscissa.

the CMV IE2 gene product increased expression of the IL-2R gene (18); however, CsA had no effect on expression of this gene, in the presence or absence of the CMV IE gene products (data not shown).

Discussion

These studies show that the CMV IE2 gene product can partially prevent the inhibitory effects of CsA on IL-2 gene transcription. Using transient transfection assays, we showed that this protective effect was more pronounced when both IE1 and IE2 were present, although IE1 alone had no effect. The observed result is expected as IE1 is known to up-regulate its own promoter, therefore resulting in increased amounts of IE2 (10, 11). The IE2 gene product also prevented the decrease in levels of steady-state IL-2 RNA that occurs in control cells in presence of CsA.

IL-2 is important for the function and expansion of T-cells (26, 27), which are the primary cells involved in foreign antigen recognition and subsequent rejection of grafted tissue (28). The ability of CsA to inhibit IL-2 production by T cells appears to be an important mechanism by which the drug prevents graft rejection (1). CsA acts primarily at the level of IL-2 gene transcription (2–4). Specifically, CsA appears to inhibit the function of nuclear binding proteins involved in transcriptional activation of the IL-2 gene (3, 4). How IE2 interferes with this inhibition is not clear. The CMV IE promoter appears to have an IE2 response element located 5′ to the gene start site (29–31); however, IE2 does not directly bind to the DNA. The IL-2 promoter does not appear to have a region of sequence homology to the area noted in the CMV promoter (data not shown). These observations suggest that IE2 alters transcription of both the CMV gene and the IL-2 gene via protein–protein interactions. Once CsA enters a cell it binds to a protein

called cyclophilin (32). It is this complex that is responsible for the immunosuppressive effects of CsA. IE2 could interfere with the binding of CsA to cyclophilin or could alter the ability of the CsA-cyclophilin complex to inhibit the functions of IL-2 binding proteins. It is also of interest, in this regard, that CsA does not alter the up-regulation of the IL-2 gene by IE2 (Fig. 3).

Interestingly, there is a subset of patients whose lymphocytes do not respond to CsA. In a study by House et al. (33), it was found that lymphocytes from some hemodialysis patients were more resistant to CsA immunosuppression that those of healthy controls. When these dialysis patients received organs, the episodes of rejection and subsequent graft failure were more prevalent in the resistant population. These results were also noted by Bowes et al. (34). It is interesting to speculate that part of the CsA resistance may have been related to the presence of a latent CMV infection. During latent infection, peripheral blood mononuclear cells (both monocytes and lymphocytes) are known to express CMV IE genes (35, 36). Therefore, IE gene up-regulation of cellular genes, such as IL-2, may also be responsible for the generation of CsA resistant lymphocyte populations.

There are additional mechanisms by which active or latent CMV infection might increase graft rejection. For example, CMV IE gene products have been shown to up-regulate HLA-DR expression (37), an effect that might result in increased recognition of foreign antigens and subsequent rejection. The IE2 gene product also appears to share sequence homology and demonstrate immunologic cross-reactivity with a region of the HLA-DR β chain (38). This type of "molecular mimicry" is thought to trigger autoimmune reactions in other types of disease (39, 40). In patients with transplanted organs and CMV infection, all of these processes may interact to enhance rejection. In addition, this study and our prior studies show that the viral gene products actually increase IL-2 gene transcription, even in the presence of CsA.

In summary, our studies show that CMV IE gene products interfere with the immunosuppressive effects of CsA on IL-2 gene transcription. These studies suggest that the CMV IE gene products may play an important role in tissue graft rejection.

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