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Interleukin-27 upregulates major histocompatibility complex class II expression in primary human endothelial cells through induction of major histocompatibility complex class II transactivator

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Summary Interleukin-27 (IL-27) is a novel IL-12 family member that plays a critical role in the regulation of T-cell responses. Its immunoregulatory effects on endothelial cells (EC) remain unexplored. Here we show a role for IL-27 in the induction of major histocompatibility complex (MHC) expression in primary human EC. Stimulation of human umbilical vein ECs by IL-27 rapidly induces IFN regulatory factor-1 and dramatically increases the expression of major histocompatibility class II transactivator (CIITA) isoform IV. Expression of this transactivator correlates with increased MHC class II gene expression. IL-27 also enhances expression of MHC class I molecules. Furthermore expression of β2-microglobulin and transporter associated with antigen processing-1 transcripts increases in response to IL-27. Additional microarray analysis demonstrates that IL-27 significantly upregulates a panel of genes that correlates with immune regulation, including the chemokines CXCL9, CXCL10, and CX3CL1 in human umbilical vein ECs. This first demonstration that both MHC II and I expression are increased in EC after IL-27 stimulation suggests that IL-27 may be important in conferring immune function on vascular endothelium.

Introduction

Major histocompatibility complex (MHC) class I and class II molecules are cell surface glycoproteins that are involved in the antigen presentation arm of the immune response. MHC II molecules are mainly expressed by professional antigen-presenting cells (APCs) and facilitate the presentation of predominantly extracellular antigenic peptides to CD4+ T cells. MHC II expression can be induced in hematopoietic and non hematopoietic cells by a number of stimuli, the most potent being interferon-γ (IFN-γ) [1,2]. Regulation of MHC II genes occurs primarily at the transcriptional level. This task is shouldered almost entirely by the class II transactivator

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ABBREVIATIONS

APCs	antigen-presenting cells
β2-m	β2-microglobulin
CIITA	class II transactivator
EC	endothelial cells
HUVECs	human umbilical vein endothelial cells
Ii	Invariant chain
IFN- γ	interferon-γ
IRF-1	IFN regulatory factor-1
MHC	major histocompatibility complex
MHC I	MHC class I
MHC II	MHC class II
TAP-1	transporter associated with antigen processing-1

(CIITA) [3,4]. The CIITA gene is controlled by at least three independent promoters known as PI, PIII, and PIV each of which generates distinct CIITA isoforms with different first exons [5]. CIITA type I is specifically and constitutively expressed in dendritic cells [5]. By contrast CIITA type III is mainly expressed by B lymphocytes, T lymphocytes, and dentritic cells [5–7]. All isoforms can be induced by IFN-γ in a variety of cell types although the major isoform is transcribed from PIV [5–9]. The expression of MHC I molecules is also essential in the immune response because they present cytosol-derived peptides to CD8+ T lymphocytes [10]. MHC I molecules are ubiquitously expressed and their level of expression can be increased by many immune cytokines.

The vascular endothelium is critically involved in the regulation of inflammation. Vascular endothelial cells (EC) are the principal cellular targets of many pro- and anti-inflammatory cytokines. They are unusually sensitive to activation by IFN-γ, showing IFN-γ-dependent activation of CIITA and upregulation of MHC I and MHC II molecules [5,11]. Enhanced expression of MHC is an immunoregulatory feature of EC activation. Recently a novel member of the IL-12 family was identified and termed IL-27 [12] that can promote both anti- and proinflammatory immune responses mainly by modulating the function of helper CD4+ T cells [13]. EC can also respond to IL-27 [14]. Because IL-27 shares many biological actions with IFN-γ, we hypothesized that IL-27 could induce CIITA and MHC gene expression in EC and regulate their APC function. This was tested by evaluating the effects of IL-27 on human umbilical vein endothelial cells (HUVECs).

Subjects and methods

Cells and materials

Recombinant human IL-27 was purchased from R&D Systems (Minneapolis, MN). EGF, bFGF, and VEGF were obtained from Preprotec (Rocky Hill, NJ) and used in conjunction with porcine intestinal heparin (Sigma, St. Louis, MO). FITC-labeled mouse mAb to HLA-DR (L423), HLA-A, B, C (G46-2.6), and isotype-control mAb were purchased from BD Bioscience (Mountain View, CA).

Human umbilical vein endothelial cells (HUVECs) were isolated from discarded umbilical cords as previously described [15], pooled, and cultured on gelatin (J. T. Baker, Phillipsburg, NJ) coated tissue culture plastic at 37°C in 5% CO_2-humidified air in Medium 199 containing 20% FBS, 2 mmol/l L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 ng/ml EGF, 5 ng/ml bFGF, 20 ng/ml VEGF, and 100 μg/ml porcine intestinal heparin. Confluent cultures were serially passaged, and cells were typically used at the second or third subculture.

RNA isolation and reverse transcription

RNA was isolated using TRIzol (Life Technologies, Rockville, MD) or Qiagen RNeasy kits (Valencia, CA), according to the manufacturer's instructions. RNA was quantitated by absorbance at 260 nm, and total RNA (2 μg) was reverse transcribed in a 40 μl reaction using 0.5 μg of random hexamer primer using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI).

Reverse transcription-polymerase chain reaction

Polymerase chain reaction (PCR) was carried out in 50-μl volumes, using 1/10–1/20 of the reverse transcription (RT) mixture (100–200 ng RNA), containing 200 μmol/l dNTPs, 50 ng of each primer, and 1.0 U *Taq* polymerase (TaKaRa Biotechonolgy, Kyoto, Japan). The PCR conditions were as follows: 5 minutes at 95°C and 21–34 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. The PCR products were run on 2% agarose gels and visualized by ethidium bromide staining. The primer sequences for HLA-A, HLA-B, HLA-C, TAP-1, and β2-microglobulin (β2-m) have been described previously [16]. Other primer sets used were: DRB, 5'-CAGCATTGAAGTCAGGTGGTTCC and 5'-CTCAGCATCTTGCTCTGTGCAG; DQA, CTCTGACCACCGTGATGAGC and 5'-CTCTCCAGGTCCACGTAGAA; DQB, 5'-CGAGTACTGGAACAGCCAGAAGG and 5'-GGAGTCATTTCCAGCATCACCAGG; DPA, 5'-CAGAGCTGTGATCTTGAGAG and 5'-AGATGCCAGACGGTCTCCTT; DPB, 5'-GGGACACAGCGCTTCCTGGAG and CAAGCAGGTTGTGGTGCTGCA; DMA, 5'-TGATCCAGCAAATAGGGCCA and 5'-CTCTGGACACCGGGATTTTC; DMB, 5'-AAAGACACCCTGATGCAGCG and 5'-TGTGGCACAATTCTGAAGCC; DOA, 5'-TGGCCCAGACCAGCTTCTAT and 5'-GGAACTTGCGGAACAAATGG; DOB, 5'-CTGTGGAGTGGAGAGCTCA and 5'-CATTACCAGACATCTGCGTC; Ii, 5'-TGGAGAACCTGCGCATGAAG and 5'-CTTGGTGGCATTCTGCATGG; and β-actin, 5'-CAGAGCAAGAGAGGCATCC and 5'-CTGGGGTGTTGAAGGTCTC. The number of cycle was evaluated for each primer set as maintaining an exponential amplification (in the linear curve): HLA-DRB, DPA and Ii, 30 cycles; DPB, DQA, DQB, DMA, DMB, and DOA, 33 cycles; DOB, 34 cycles; HLA-A, B, C, and β2-m, 25 cycles; TAP-1, 29 cycles; and β-actin, 21 cycles.

Real-time RT-PCR

For real-time PCR, primers were designed using Primer Express software (Applied Biosystems, Foster City, CA). The primer pairs for CIITA isoforms I, III and IV have been described previously [17]. Other primer sets used were: DRA, 5'-GCTCTGAGTGGCGAAATCAAG and 5'-CAATGCTAGGTACTGCGGGAG; IRF-1, 5'-AAGGATGCCTGTTTGTTCCG and 5'-CAGCGAAAGTTGGCCTTCC; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-TGGGCTACACTGAGCACCAG and 5'-GGGTGTCGCTGTTGAAGTCA. The 2x SYBR Green PCR Master Mix (Applied Biosystems) was used in PCR with 200 nmol/l of forward and reverse primers, 10–40 ng of the reverse-transcription product, and RNase/DNase-free water to 50 μl. The PCR mixtures were transferred to MicroAmp optical 96-well reaction plates and run on the Applied Biosystems GeneAmp 7500 Sequence Detection System under the following conditions: 10 minutes at 95°C and 40 cycles of 94°C for 15 seconds, 60°C for 30 seconds, and 72°C for 34 seconds. Relative quantities of the gene of interest were determined for unknown samples by the comparative threshold cycle (delta CT) method and normalized to GAPDH quantities. To determine the