Letter to the Editor

Expression of CXCL16 in Human T Cells

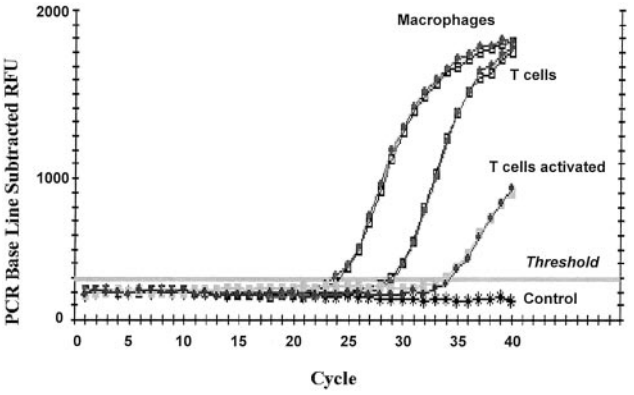
To the Editor:

In the November 2001 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Minami et al[1] demonstrated expression of a novel scavenger receptor for phosphatidylserine and oxidized lipoprotein (SR-PSOX), which is identical to the chemokine CXCL16, in lipid-laden macrophages accumulated in the intima of atherosclerotic lesions. Based on reports of this group as well as reports of other authors,[2–4] SR-PSOX expression has been shown to be a unique feature of antigen-presenting cells (macrophages, dendritic cells, and CD19-positive B lymphocytes) but not of other cells. However, in the April 2002 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Hofnagel et al[5] used reverse-transcription–polymerase chain reaction (RT-PCR) to detect expression of SR-PSOX in smooth muscle cells and endothelial cells, thus raising the question whether SR-PSOX/CXCL16 may be expressed in other cells besides antigen-presenting cells. These authors also observed a lack of regulation of this ligand by tumor necrosis factor-α and by other proinflammatory stimuli, calling into question the mechanisms activating CXCL16 expression.[5]

Using quantitative real-time RT-PCR, we found expression of CXCL16-specific mRNA in human T cells. Human peripheral blood was collected from antecubital vein of healthy donors. White blood cells were isolated by centrifugation on Histopaque 1.077. Monocyte-derived macrophages (M) were isolated by 1-hour adherence to plastic,[6,7] while nonadhering cells were applied to a T cell enrichment column (R&D Systems, Inc.). The purity of M and the purity of T cells were greater than 85% to 95% as estimated by flow cytometry with anti-CD14 IgG and anti-CD3 IgG, respectively. The T cell fraction did not contain CD19- or CD14-positive cells. T cells were activated with phorbol myristate acetate (PMA) and ionomycin in the presence of monensin. Activation was confirmed by expression of CD69 as well as by intracellular staining for IFNg and interleukin (IL)-4. Activation of M was verified morphologically and by production of reactive oxygen species.[8]

Real-time one-tube RT-PCR (primers for human CXCL16: 5′-TCTCAAAGAATGTGGACATGC-3′ and 5′-CAGGGGTGTGGA-TATCTGAA-3′) was performed on total RNA isolated by using TRIzol reagent (GIBCO BRL). After the reverse transcription step, PCR was performed at 94°C (15 seconds), 58°C (30 seconds), and 72°C (30 seconds) with the data collection step at 79.5°C (15 seconds) for 40 cycles. Specificity of product was confirmed by analysis of characteristic melting curve and by electrophoresis. The sequence of the CXCL16 product was confirmed by sequence analysis. Negative controls were performed by omitting the reverse transcription step and by RNAse treatment. DNAse I treatment did not change the signal, demonstrating an absence of genomic DNA in RNA preparations. GAPDH served as a control.

Real-time RT-PCR demonstrated the expression of CXCL16 mRNA in human T cells as well as in human M, which were used as a positive control (Figure). We have detected CXCL16 message in 5 of 6 tested samples of isolated human T cells (Table). The level of CXCL16 expression in T cells was found to be similar to that in the human monocytic cell lines U937 and MM6 (5.5% against 2.8% and 9.7%, respectively). The mean level of CXCL16 expression in T cells was 10.6±6.0% of the level found in M of the same donors (2.1% to 22.2%, n=3). This fact suggests that CXCL16 mRNA expression in T cells is significantly less than in macrophages and comparable with that in monocytic cell lines. Activation of M and T cells with PMA/ionomycin led to strong downregulation of CXCL16 expression, with more profound effect in M (Figure, Table). This effect is gene-specific, as demonstrated by the fact that GAPDH is not attenuated in stimulated cells (data not shown).



Results of a representative real-time RT-PCR for CXCL16.

T cells express the receptor for CXCL16, termed CXCR6 or Bonzo.[3,4,6,9] Our data show that T cells also express the CXCR6 ligand CXCL16. There are well-known examples of expression in the same cell of both ligand and receptor, such as IL-2 and IL-2 receptor in T cells.[10] Evidently, this represents one of the mechanisms for autocrine stimulation of T cells. The current finding is the first observation of a similar pattern of expression for the CXC chemokine CXCL16. Here we demonstrate that T cells also express CXCL16 at a level one order less than in macrophages, comparable with that of monocytic cell lines, and that activation of T cells with PMA/ionomycin downregulates the CXCL16 message. Further experiments are needed to investigate whether both CXCL16 and CXCR6 are expressed on the same T cells or on different subsets of CD3-positive cells and to investigate the functional role of possible co-expression.

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CXCL16 Expression in Human Macrophages and T Cells and Effect of Activation With PMA/Ionomycin

	Macrophages		Effect of	T Cells		Effect of
	Nonactivated	Activated	Activation	Nonactivated	Activated	Activaton
Cycle No.	24±0.7	29±0.3		29±0.9	33±1.4	
CXCL16, %	100	6.4±1.5	−94%	5.5±2.5	1.6±0.9	−72%
Range, %	—	4.2–9.3		0–16.3	0–5.3	
N	4	3		6	6	

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