# Expression of interleukin-6 receptor on blood lymphocytes without *in vitro* activation

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## SUMMARY

A monoclonal antibody against the interleukin-6 receptor (IL-6R) has been used in a high-sensitivity immunofluorescence technique to study receptor expression on unstimulated blood lymphocytes. Most CD4 cells express IL-6 receptor, whilst a small and variable proportion of CD8 and B cells are positive. $CD4^+$ cells express higher levels of receptor than $CD8^+$ T cells, and $CD45RO^+$ cells express higher levels than CD45RA cells.

Interleukin-6 (IL-6) is a cytokine produced by a variety of cells of both lymphoid and non-lymphoid origin, and active in a variety of tissues and cells, both within and outside the immune system.[1,2] The expression of low levels (a few hundred molecules per cell) of IL-6 receptor by resting T (but not B) cells has been demonstrated by binding studies with radiolabelled IL-6.[3] Hirata *et al.*[4] prepared a monoclonal antibody (MT18) and used it to study IL-6 receptor (IL-6R) expression on resting and activated lymphocytes. The low levels of expression on unstimulated cells meant that the labelled and control fluorescence histograms overlapped extensively, and it was not possible to draw conclusions on the proportions of cells in each subset expressing the receptor. We have developed a procedure for the detection of markers present at low concentrations, capable of detecting as few as 100 molecules/cell,[5] and have demonstrated the presence of IL-2R p55 and p75 chains on unstimulated blood lymphocytes.[6,7] In this study, the monoclonal antibody MT18 and the high-sensitivity procedure are used to examine the expression of IL-6R on unstimulated T and B cells, and to determine the expression of the receptor by subsets of cells.

The IgG2b monoclonal antibody MT18, directed against the IL-6R, has been characterized previously.[4] Purified antibody was kindly provided by Dr T. Taga and Professor T. Kishimoto, (Osaka University, Osaka, Japan). MT18 antibody was used at 6 $\mu$g/ml, adding 25 $\mu$l to $5 \times 10^5$ cells in 50 $\mu$l. IL-2R p55 (CD25, TAC) was detected with the 7G7B6 monoclonal antibody.[8] For two-colour analysis the following fluorescein-conjugated antibodies were used; CD3: Leu-4a; CD4, Leu-3a; CD8, Leu-2a; CD20, Leu-16; CD45RA, Leu-18; all from Becton Dickinson (San Jose, CA); CD45RO, UCHL1 from Dako (Glostrup, Denmark). Antibody was detected by biotinylated horse anti-mouse Ig (Vector Laboratories, Burlingame, CA) followed by phycoerythrin-streptavidin (PE-SA, Caltag Laboratories, San Francisco, CA), as described previously.[5,7] In some experiments normal horse serum (20 $\mu$l) and normal human IgG (5 $\mu$l, 160 $\mu$g/ml) were added, to reduce non-specific staining. Samples were analysed on a FACScan instrument (Becton Dickinson).

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Figure 1 shows the fluorescence histogram for peripheral blood lymphocytes (PBL) reacted with MT18 antibody and the isotype-matched negative control. Fifteen samples were analysed, giving a mean value of 47% positive (SD = 14). Occasional high values (including one of 81% on a donor who usually gave values around 40%) suggested that IL-6R expression may change dramatically in response to factors such as infection. Dual-parameter analysis showed 48% of $CD3^+$ T cells reacting with the MT18 antibody (Fig. 2). The majority of $CD4^+$ cells were positive (87–98%, seven donors); a smaller proportion of $CD8^+$ cells (20–54%, seven donors) expressed IL-6R. The $CD4^+$ cells showed brighter staining with MT18 than CD8 cells (Fig. 2). B cells were either negative (Fig. 2), or showed a small proportion of positive cells (up to 30%, seven donors). For the sample illustrated in Fig. 2, of the $MT18^+$ cells 63% were $CD4^+$ and 28% were $CD8^+$. Using CD3 as a marker, 95% of $MT18^+$ cells were T cells. Cells expressing the p180 form of the leucocyte-common antigen (CD45RO) showed brighter staining with MT18 than did $CD45RA^+$ cells (Fig. 2). Sixty-one per cent of $CD45RO^+$ cells and 28% of CD45RA cells were $IL\text{-}6R^+$, suggesting that IL-6R expression is more common among antigen-experienced (memory) cells than among naive cells.

Expression of CD25 (TAC, p55 chain of the IL-2R) was examined in parallel with IL-6R expression. This marker is predominantly expressed on CD4 and B cells.[6] A proportion at least of CD4 cells and CD45RO cells must co-express IL-2 and IL-6 receptors (Table 1). Indeed, apart from B cells, the similarity in proportions suggests that both receptors may mark predominantly memory (CD45RO) CD4 cells. To further analyse co-expression of IL-6R and IL-2R-p55, cells were

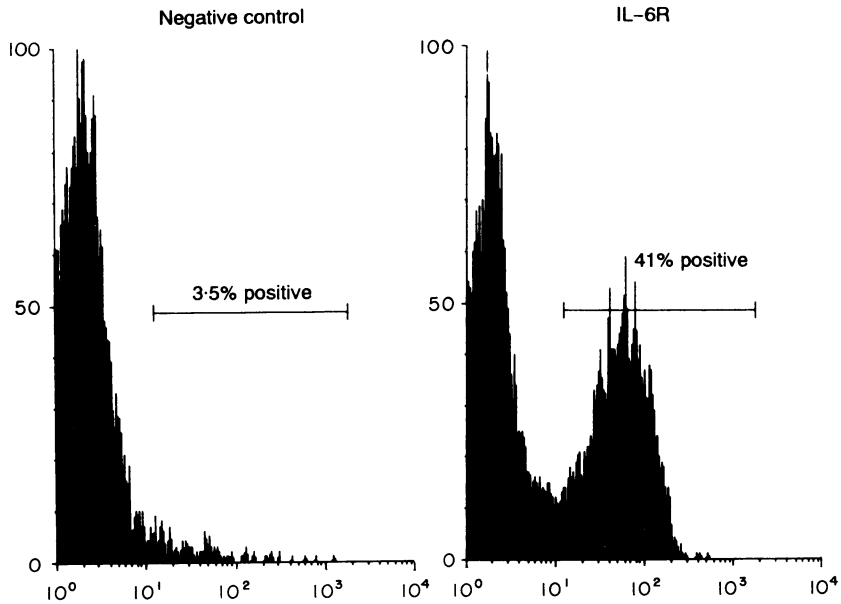


**Figure 1.** Fluorescence histogram for MT18 staining of normal blood mononuclear cells (gated to exclude monocytes), and isotype-matched negative control.

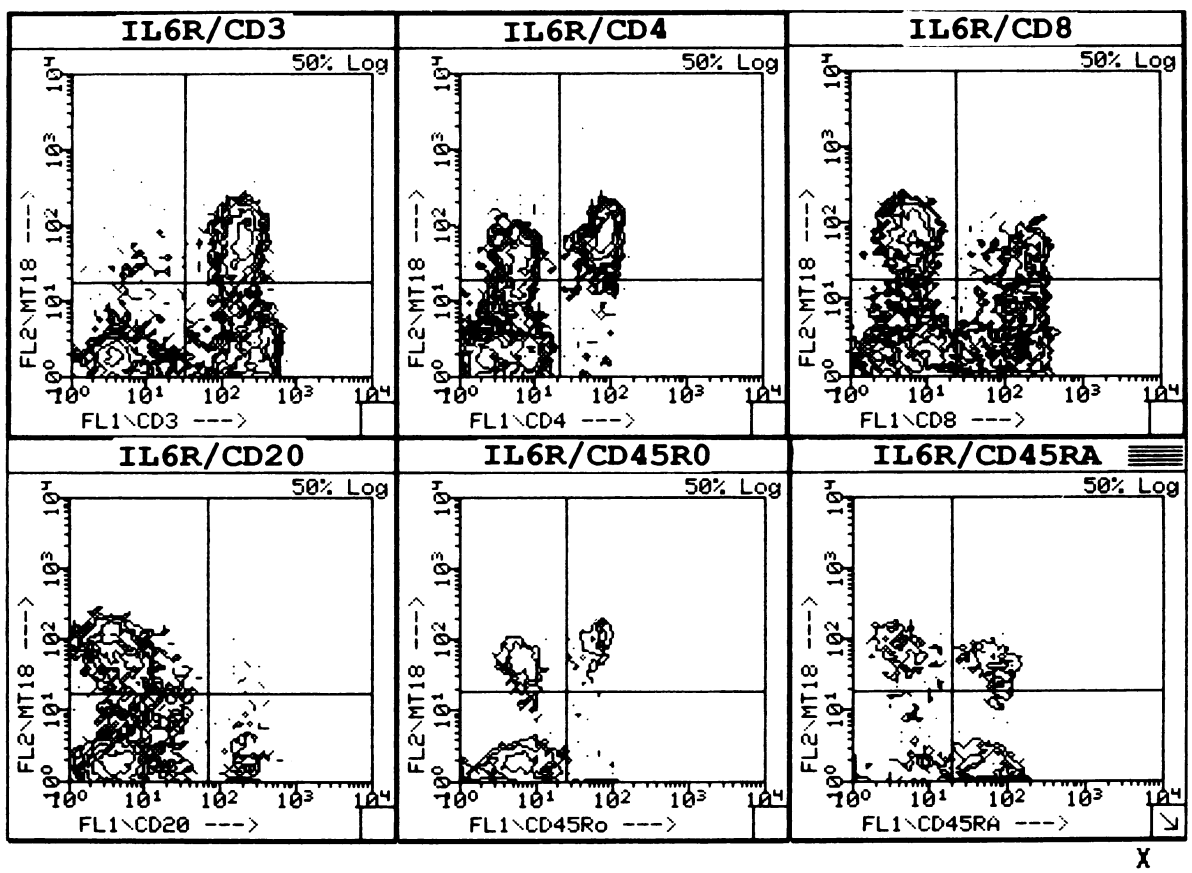


**Figure 2.** Contour plots for MT18 staining of T cells (CD3), T-cell subsets (CD4, CD8), B cells (CD20) and CD45 isoforms (CD45RA and CD45RO). CD4 cells are the predominant IL-6R-expressing population, and IL-6R is expressed preferentially in CD45RO rather than CD45RA cells.

**Table 1.** Comparison of expression of IL-6R and IL-2R-p55

| Cell population (gated) | % positive | |
|---|---|---|
| | IL-6R | IL-2R-p55 |
| PBL | 35 | 26 |
| CD3 | 48 | 30 |
| CD4 | 87 | 61 |
| CD8 | 29 | 19 |
| CD20 | 7 | 75 |
| CD45RO | 61 | 68 |
| CD45RA | 28 | 19 |

labelled with CD25 antibody and sorted to collect the negative cells. Because of the low level of expression of CD25, the negative and positive peaks were not resolved fully and significant numbers of stained cells were recovered in the negative fraction. Nevertheless, depletion of $CD25^+$ cells was accompanied by depletion of $IL\text{-}6R^+$ cells, supporting the conclusion that at least a substantial proportion of cells co-express the two receptors.

Since IL-6R expression may be modulated in diseases involving the immune system, we studied a small number of patients with human immunodeficiency virus (HIV) infection and a few cases of lymphoid malignancy, to see if a broader examination of IL-6R expression as a disease marker is warranted. In 10 patients with HIV infection, the majority of CD4 cells were $IL\text{-}6R^+$, and B cells were largely $IL\text{-}6R^-$; in both respects not different from controls. Breen *et al.*[9] have shown elevated levels of IL-6 in serum from patients infected with HIV, and have suggested that this is connected with the polyclonal B-cell activation seen in these patients. If high levels of IL-6 are causing, rather than reflecting, cellular activation, one might expect to see increased numbers of B cells expressing IL-6R. Clearly a larger study, specifically identifying patients with polyclonal B-cell activation, is needed. Six CLL samples were studied, and none expressed detectable levels of IL-6R. One sample of plasma cell leukaemia did react with the MT18 antibody, in accord with the proposed role of IL-6 as a growth factor for plasma cells.[1]

The results demonstrate expression of the IL-6R by 30–50% of unstimulated blood lymphocytes, including the majority of $CD4^+$ T cells and a smaller and variable proportion of $CD8^+$ cells and B cells. The use of a high-sensitivity staining procedure allows these cells to be distinguished quite clearly from $IL\text{-}6R^-$ cells. What is the functional significance or IL-6R expression by these cells? IL-6 has a co-stimulatory role in the activation of T cells (reviewed in ref. 2) Of particular interest is the finding that IL-6 can induce expression of the IL-2R.[10,11] van Snick[2] has suggested that the separate effects of IL-1 and IL-6, which lead to proliferation that is inhibited by anti-IL-2, are best explained if IL-1 up-regulates IL-2 production and IL-6 up-regulates IL-2R. Since our results show that many CD4 cells express both IL-6R and IL-2R/p55, but CD4 cells are generally $IL\text{-}2R/p75^-$,[7] we speculate that IL-6 may up-regulate p75 expression on CD4 cells, thus making them responsive to physiological concentrations of IL-2.

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