EXPRESSION OF IL-2 RECEPTOR ß-CHAIN IN B16 MELANOMA CELLS

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The activity of Interleukin-2 (IL-2), the main T cell growth factor, has been thought to be restricted to the inmune system, however certain nonhematopoietic cells have been shown to respond to IL-2 and/or to express its receptor (IL-2R) (Benveniste and Merrill, 1986; Reinecker and Podolsky, 1995). In addition, the expression of IL-2R has been reported in several tumor cells of nonhematopoietic origin such as adenoma (Artz et al, 1992)), squamous cell carcinoma (Weidmann et al, 1992)) and melanoma (Plaisance et al, 1993). Although the biological significance of the presence of IL-2R in tumor cells remains unclear it has been suggested that IL-2 could modulate the expression of several molecules implicated in tumor progression and metastasis.

The cell membrane receptor for IL-2 consists of at least three protein molecules, α subunit (p55), ßsubunit (p70-75) and γ subunit (p64) which form a functional complex. In previous investigations we have demostrated that murine B16F10 melanoma cells express the α chain of IL-2R. Moreover the IL-2R on B16F10 cells was functional as cells increased their proliferative activity when cultured in the presence of IL-2. The effect of IL-2 on cell proliferation suggested that IL-2Rß and/or IL-2Rγ should also be present in B16 melanoma cells as these subunits are involved in signal transduction while IL-2R by itself is not able to deliver the signal to the cell cytoplasm. So to confirm this we have analyzed the expression of the ß chain of IL-2R in B16 melanoma. Furthermore, considering the possible implication of IL-2 in tumor progression we wanted to know if the expression of this molecule could be different in cells of different metastatic ability; so we have studied IL-2R expression in the parental line of B16 melanoma and in two metastatic variants (B16F1 and B16F10).

The expression of IL-2Rß on the cell surface was determinated by flow cytometry analysis. For the indirect immunofluorescence assay, B16 melanoma cells, (parental, F1 andd F10 sublines) were obtained from adherent cultures by EDTA treatment and after washing cells were incubated first with anti-IL-2Rß monoclonal antibody (Endogen) and then with FITC-conjugated rabbit anti-rat IgG (Dakopatts). After inmune staining cells were analyzed using a Coulter EPICS 752. Both the percentages of IL-2R positive cells and the mean of fluorescence intensity were determined by Immunotest analysis (Coulter). CTLL-2 cells were included in all experiments as positive control, as these cells to proliferate depend on the presence of IL-2 in the culture medium and express constitutively IL-2 receptors. The cytometric analysis showed that the three B16 melanoma sublines were reactive for anti-IL-2Rß antibody (Table 1).

Table 1. Flow cytometric analysis of IL-2Rβ expression on metastatic variants of B16 melanoma cells

	positive cells (%)	fluorescence intensity
CTLL-2	22.23 ± 15.80	43.16 ± 15.15
B16F0	12.19 ± 3.00	79.77 ± 13.29
B16F1	7.18 ± 2.17	78.56 ± 20.70
B16F10	7.37 ± 1.06	87.55 ± 13.38

The mean values ± SD of three independent experiments are shown.

The percentage of cells displaying surface staining after anti-IL-2Rß immunolabelling was similar in the two metastatic variants tested (B16F1 and B16F10) and a little higher in the parental B16. There were minor differences in the level of expression of IL-2Rß on B16 cells between experiments. In contrast in CTLL-2 cells the percentage of positive cells ranged from 10 % to 54 %. Moreover, the population of B16 cells which expressed the IL-2Rß when compared with CTLL-2 showed higher intensity of fluorescence (FI) which means that the number of receptors per cell was higher in B16 cells than in the lymphoid cells. From these results we concluded that IL-2Rß is constitutively expressed in B16 melanoma cells, being the level of expression low and without correlation with the metastatic ability of the tumor cells.

In addition to the cytometric analysis of IL-2Rß on the cell surface of B16 melanoma cells we have analyzed the expression of IL-2Rβ mRNA. The presence of the message for the ß subunit of IL-2R was assesed by reverse transcription followed by polimerase chain reaction (RT-PCR assay). Total cellular RNA was obtained from B16F10 cells and subjected to reverse transcription using oligo(dT)12-18 (Clontech) as a primer and recombinant reverse transcriptase of the Moloney murine leukemia virus (USB). Then, the reverse transcribed mixture was subjected to 40 cycles of amplification in the presence of Taq polymerase (Perkin-Elmer/Cetus). In all RT-PCR experiments, ß-actin was included. The primers used to amplify the IL-2Rß mRNA sequence were 5'- CTC CGT GGA CCT CCT TGA CAT AAA TGT GG-3' and 5'-TGT TTC GTT GAG CTT TGA CCC TCA CCT GG-3' and the primers used for the ß-actin mRNA amplification were 5'-GTG GGC CGC TCT AGG CAC CAA-3' and 5' CTC TTT GAT GTC ACG CAC GAT TTC-3' (mouse MAPPing™ Amplimers, Clontech). The sizes of the amplified products were 348 bp for IL-2Rß and 540 bp for ß-actin. After amplification, electrophoresis of the PCR reaction mixture was performed and gels were stained and photographed. Control PCR without cDNA and control positive fragment were also included to exclude contamination.

As it can be observed in figure 1 the amplified product from B16F10 cells had identical mobilitiy on agarose gels than the positive control (Fig. 1A, lane 2 and lane 3, respectively). In these two lanes it can be seen a band whose size corresponds to a DNA fragment of 348 bp as it is deduced when comparing with molecular weight markers (lane 1); which in turn corresponds to the amplified product of IL-2Rß. So it was also desmonstrated at the molecular level that B16 melanoma cells express IL-2Rß.

As we have just shown, B16 cells express IL-2Rß without prior stimulation. The expression of the α chain in B16 melanoma was constitutive too, however it could be modulated by several factors. In particular we observed that Cyclosporin A (CsA), a potent immunosuppresive agent that inhibits the translation of several genes codifying for interleukins and their receptors, induced an increment in the expression of IL-2Rα in B16F10 cells both at the mRNA and the protein level. Furthermore, the same treatment of tumor cells "in vitro" induced an increment in the metastatic colonization of the liver.

At the moment we do not know if the upregulation of the alfa chain of IL-2R and the prometastatic effect of CsA are related events; nevertheless we considered of interest to asses the effect of CsA on the beta chain of the receptor.

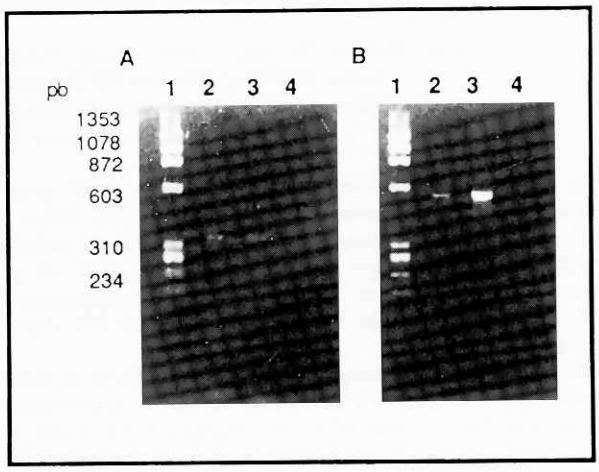

Figure 1. Analysis of IL-2Rβ mRNA expression in B16F10 melanoma cells. (A) IL-2Rβ (B) β-actin. Lane 1: molecular weight markers (øX174/Hae III). Lane 2: B16F10. Lane 3: positive control. Lane 4: negative control.

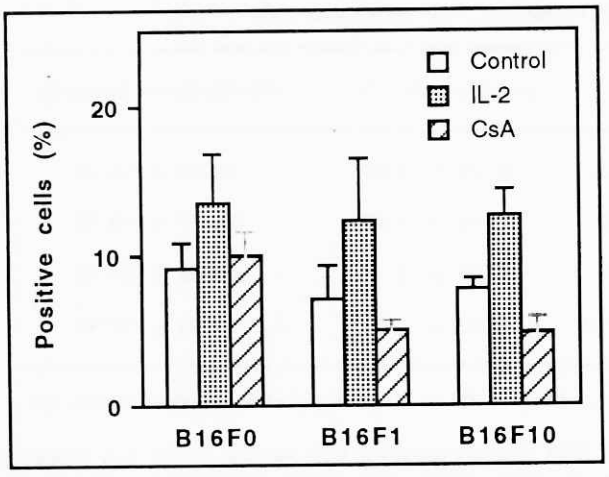

Figure 2. Effect of IL-2 and CsA treatments on the expression of IL-2Rβ in three sublines of B16 melanoma.

In addition to CsA treatment we have also performed the analysis of IL-2R with B16 cells cultured in the presence of IL-2. IL-2 has been termed to upregulated the expression of its own receptor. The upregulation of IL-2R could be relevant considering that *in vitro* treatment of B16F10 cells with IL-2 leads to increase formation of metastases in the liver.

To determine the modulation of IL-2Rß by CsA and IL-2 we have cultured the three sublines of B16 melanoma in the presence of CsA (5 μg/ml) or IL-2 (500 U/ml), for 24 h, prior to FACS analysis. Results from these experiments are shown in figure 2. It can be observed that, in contrast with the effect induced on the expression of the α subunit, the expression of IL-2Rß in B16F1 and B16F10 sublines was lower after treatment with CsA than in untreated cells. However this reduction was not observed in the parental line. When cells were treated with IL-2 the expression of IL-2R augmented: the percentage of cells stained was significatively higher in the three sublines tested. The increment induced by IL-2, although statistically significative, was small, raising the number of positive cells from aproximately 7 % of stained cells in controls (B16F1 and B16F10) to 12 % in IL-2 treated cells. So only one of the prometastatic treatments used induced a positive modulation of IL-2Rß.

Taking all these results into account we conclude that the ß subunit of the IL-2R is present in B16 melanoma cells although there seems to be no correlation between the level of expression of IL-2Rß and the metastatic ability of B16 melanoma cells, as it can be deduced from the facts that the expression was similar in the metastatic variants of B16 and that only one of the treatments which increased the liver colonization by B16 cells (IL-2 treatment) resulted in a higher expression of this molecule. Nevertheless the proliferative effect of IL-2 on B16 cells detected in previous experiments suggests that the expression of IL-2R, in certain conditions, could be advantageous for the tumor cells in regard to their metastatic activity.

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