**Research article**

**Interleukin-2 and its receptor complex (α, β and γ chains) in *in situ* and infiltrative human breast cancer: an immunohistochemical comparative study**

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Received: 19 Jun 2003   Revisions requested: 14 Aug 2003   Revisions received: 8 Sep 2003   Accepted: 1 Oct 2003   Published: 22 Oct 2003

Breast Cancer Res 2004, 6:R1-R7 (DOI 10.1186/bcr730)

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**Abstract**

**Introduction.** The presence and distribution of interleukin-2 (IL-2) and its receptor complex (Rα, Rβ, Rγ) were studied in 52 women who were clinically and histopathologically diagnosed with breast tumours (17 *in situ* and 35 infiltrating), and in 13 women with benign fibrocystic lesions in the breast.

**Methods.** Immunohistochemistry with antibodies against IL-2, IL-2Rα, IL-2Rβ and IL-2Rγ was used. A comparative semiquantitative immunohistochemical study between the three breast groups (fibrocystic lesions, *in situ* tumours and infiltrating tumours) was performed.

**Results.** IL-2 and its three receptor chains were immunodetected in the cytoplasm of epithelial cells. The three receptor chains were also detected on the cell surface. In fibrocystic lesions, immunoreactions to IL-2 (38.5% of cases), IL-2Rα (53.8%) and IL-2Rβ (30.8%) were very weak, whereas immunoreaction to IL-2Rγ (46.1%) was somewhat more intense. In *in situ* tumours, the percentages of cases that immunostained positively for IL-2 and its three receptor chains were similar to those observed in fibrocystic lesions, but immunostainings of the four antibodies were more intense. In infiltrative tumours, the percentages of positively stained cases and also immunostaining intensities were approximately twice that found for *in situ* tumours. Within infiltrating tumours, the percentage of cases showing immunoreaction to IL-2 and their three receptor chains was higher in the patients with lymph node infiltration at the time of surgery.

**Conclusion.** The development of breast tumour is associated with an increased expression of IL-2 and its three receptor chains, and this expression also seems to be associated with the malignancy of the tumour.

**Keywords:** breast cancer, interleukin-2, interleukin-2 receptor
In normal tissues [7] and several tumours, including stomach, renal and spinocellular cancer [8] and squamous cell carcinomas of the head and neck [9], IL-2 seems to stimulate cell proliferation and is more abundant in mitotic cells than in cells in the other phases of the cell cycle [8,9]. Experimental studies of squamous cell carcinomas of head and neck have shown that antisense IL-2 treatment or blocking of IL-2Rβ results in growth inhibition [12].

Nevertheless, it has also been shown that systemic administration of IL-2 induces the regression of metastatic murine renal carcinoma [13], possibly through the induction of interferon-γ, tumour necrosis factor-α and anti-angiogenic products such as IP-10 [14]. This finding has led to some authors to conclude that whereas endogenous IL-2 stimulates cell proliferation, exogenous IL-2 inhibits such a proliferation [7,15].

Studies on IL-2 and its receptors in breast cancer are few. Katano and colleagues [16] cultured two breast carcinoma cell lines (M and M25-SF lines), and found IL-2 expression in the latter line only. In vivo immunohistochemical studies of IL-2Rα and IL-2Rβ in ducal infiltrating breast cancer by Ridings and colleagues [10] revealed that only the β receptor chain was present in these tumours.

The aim of the present study was to characterise the expression patterns of IL-2 and its three receptor chains (α, β and γ) by immunohistochemistry, in both in situ and infiltrating breast tumours, to elucidate the role of these proteins in breast cancer progression and metastasis.

Materials and methods

Total or partial mastectomy specimens obtained from 52 women, who were clinically and histopathologically diagnosed with breast adenocarcinoma during 1998 in our hospital, were used for the study. Seventeen of these women (aged from 47 to 75 years) presented in situ carcinoma (8 lobular and 9 ductal); 1 of these 17 women also showed lymph node infiltration at the time of surgery. Thirty-five women (aged from 51 to 77 years) had infiltrating carcinoma (15 lobular and 20 ductal); 13 of these 35 women showed lymph node infiltration at the time of surgery, and 7 of these 13 developed metastasis 7–24 months after surgery. At present (January 2003), neither the remaining 22 women with infiltrating tumours nor the 17 women with in situ tumours have developed metastasis.

Tumour samples were compared with breast biopsies from 13 women (aged from 16 to 59 years) with benign fibrocystic lesions. Removal of tissues and the study of samples were approved by the Hospital’s Ethics Committee and were performed with the consent of the patients’ relatives.

All samples were processed for immunohistochemical study by light microscopy. The primary antibodies used were goat anti-human IL-2, rabbit anti-human IL-2Rα, rabbit anti-human IL-2Rβ and rabbit anti-human IL-2Rγ (Santa Cruz Biotechnology, California, CA, USA).

Tissues were fixed for 24 hours at room temperature in 0.1 M phosphate-buffered 10% formaldehyde, then dehydrated and embedded in paraffin. Sections (5 µm thick) were processed with the avidin–biotin–peroxidase complex (ABC) method. Following deparaffination, sections were hydrated and then incubated for 30 minutes in 0.3% H2O2 diluted in methanol to reduce endogenous activity. After rinsing in Tris-buffered saline (TBS), the slides were incubated with normal donkey serum at 10% in TBS for 30 minutes, to prevent non-specific binding of the first antibody. Thereafter, the primary antibodies were applied at a dilution of 1:100 for the four antibodies in TBS overnight at room temperature. The sections were then washed twice in TBS and incubated with rabbit anti-(goat IL-2), or pig anti-(rabbit IL-2Rα, IL-2Rβ and IL-2Rγ) biotinylated immunoglobulin (Dako, Barcelona, Spain) at 1:500 in TBS. After incubation for 1 hour with secondary antibody, the sections were incubated with a standard streptavidin–biotin complex (Vector Laboratories, Burlingame, CA, USA) and developed with 3,3′-diaminobenzidine (DAB), using the glucose oxidase–DAB–nickel intensification method.

Immunohistochemical procedure specificity was checked with negative and positive controls. For negative controls of immunoreactions, tissues of each type were incubated with preimmune serum at the same immunoglobulin concentration as that used for each antibody, or with blocking peptides (Santa Cruz Biotechnology). As positive controls, histological sections (immunohistochemistry) of thymus samples were incubated with the same antibodies.

A comparative histological quantification of immunolabelling among the different groups of breast samples (in situ adenocarcinomas, infiltrating adenocarcinomas and benign fibrocystic lesions) was performed for each of the four antibodies. For each breast sample, six histological sections were selected at random. In each section, the staining intensity (optical density) per unit surface area was measured with an automatic image analyser (MIP4 version 4.4; Consulting Image Digital, Barcelona, Spain) in five light microscopic fields per section, using the 40× objective. Delimitation of stained surface areas was performed manually with the mouse of the image analyser. For each positively immunostained section, one negative control section (the next in a series of consecutive sections) was also used, and the optical density of this control section was subtracted from that of the stained section. From the average values obtained for each breast, the means±SD for each breast group were calculated. The statistical significance between means of the different
breast group samples was assessed by the Fisher and Behrens test at $P \leq 0.05$, by multiple pairwise comparisons of all the values for each breast zone, for each specific antibody separately.

To test whether the increase in IL-2 immunoexpression intensity (optical density) was correlated with the increase in expression of each IL-2 receptor chain, Pearson’s coefficient of correlation was calculated for each of the following pair of values (IL-2 and IL-2Rα, IL-2 and IL-2Rβ, IL-2 and IL-2Rγ).

Results

No immunoreaction was observed in the negative controls incubated with preimmune serum or with the use of the antibodies preabsorbed with an excess of purified antigen.

IL-2 immunostaining. (a) Thymus sections were used as a positive control. Fibrocystic lesions show either no immunoreaction (b) or a weak immunoreaction limited to a circumscribed supranuclear region (c). In ductal in situ (d) and ductal infiltrating (e) carcinomas, most of the cytoplasm was labelled. Original magnification $\times 375$.

Staining of thymus sections (positive controls) was always positive for all antibodies used (Figs 1a, 2a, 3a and 4a).

In the three groups of specimens (fibrocystic lesions, in situ tumours and infiltrating tumours), immunostaining of IL-2 appeared in the cytoplasm of epithelial cells, whereas immunostaining of the three types of IL-2R (α, β and γ) appeared in both the cell surface and cytoplasm of epithelial cells.

For each antibody assayed, the percentage of positive cases and immunostaining intensities are shown in Table 1. The immunostaining intensities (optical density) are given as means ± SD. Significance was determined by multiple pairwise comparisons by the Fisher and Behrens test at $P \leq 0.05$. All the values for each breast zone and for each specific antibody were compared separately.

Immunoreaction to IL-2 was found in 38.5% of benign fibrocystic lesions (Fig. 1b,c), in 41.2% of in situ tumours (Fig. 1d) and in 82.9% of infiltrating tumours (Fig. 1e).
Immunostaining was very weak in benign fibrocystic lesions, and more intense in both tumour types.

Immunoreaction to IL-2Rα was present in 53.8% of fibrocystic lesions (Fig. 2b), in 35.3% of in situ tumours (Fig. 2c) and in 60.0% of infiltrating tumours (Fig. 2d). Immunostaining was very weak in benign fibrocystic lesions, more intense in in situ tumours, and even more intense in infiltrating tumours.

Immunoreaction to IL-2Rβ was observed in 30.8% of fibrocystic lesions and was very weak (Fig. 3b). An intense immunoreaction was detected in 35.3% of in situ tumours (Fig. 3c) and in 80.0% of infiltrating tumours (Fig. 3d).

Immunoreaction to IL-2Rγ appeared in 46.1% of fibrocystic lesions. This was the only IL-2 receptor that showed a certain degree of staining intensity in fibrocystic lesions (Fig. 4b). Immunoreaction to this receptor was observed in 41.2% of in situ tumours (Fig. 4c) and in 80.0% of infiltrating tumours (Fig. 4d).

Within the group of infiltrating carcinomas with lymph node infiltration (13 women), 10 of them (76.9%) presented a positive immunoreaction to IL-2 and its three receptor chains. In the group without lymph node infiltration (22 women), only 10 cases (45.5%) presented a positive immunoreaction to IL-2 and its receptors.

The correlation (r) between the immunoexpression intensity of IL-2 and each receptor chain was as follows: IL-2/IL-2Rα, 0.88; IL-2/IL-2Rβ, 0.97; IL-2/IL-2Rγ, 0.85.

Discussion

Immunohistochemical studies of IL-2 and IL-2R have been performed in several normal tissues such as human and murine fibroblasts [17,18], oral mucosae [9] and epithelial basal cells of the prostate [11], and it has been suggested that IL-2 is involved in the control of the equilibrium between proliferation and apoptosis [11]. In all these normal tissues the expression of IL-2 and IL-2R was weak. These data agree with present findings in fibrocystic lesions of the breast. In these specimens, breast epithelial cells showed a characteristic staining pattern: a weak immunoreaction to IL-2, limited to a circumscribed cytoplasmic area corresponding to the Golgi zone. These observations suggest that, in breast tissue, this interleukin is processed and secreted in the same way as in haematopoietic cells, in agreement with the report by Sander and colleagues [19].

The functional location of IL-2 receptor complex is the plasma membrane. Each of the three chains has a cytoplasmic domain [20], which binds to intracellular signalling molecules and thereby relays specific intracellular signals [21]. In the present study, the three IL-2R chains were immunodetected not only at the cell surface but also in the cytoplasm. This intracellular immunoreaction might correspond to neosynthesised receptor chains that have not yet reached the cell membrane.

During the past decade, consistent results have demonstrated the occurrence and functional implication of IL-2 in tumour cells [7,9]. In the present study, in contrast with the weak immunolabelling observed in benign fibrocystic lesions, breast tumour cells (in situ tumours, and even...
more in infiltrating tumours) showed a large part of the cytoplasm immunostained (from moderately to intensely) to IL-2 and its receptor. According to the staining distribution there are no specific subpopulations within individual patients, because IL-2/IL-2R expression was uniform throughout the whole epithelium. This suggests that IL-2 secretion acts throughout an autocrine mechanism. There also was a positive correlation between the increased expression of IL-2 and that of its three receptor chains: IL-2Rα \( r = 0.88 \), IL-2Rβ \( r = 0.97 \) and IL-2Rγ \( r = 0.85 \). Nevertheless, more than half of in situ carcinoma patients, and about 20% of patients with infiltrating carcinomas, did not immunoreact to IL-2 and IL-2R. The association between IL-2 and breast cancer might therefore not be generalised.

The effect of this high expression of IL-2 and IL-2R in breast cancer cells might be a contribution to the development of a tumour by enhancing cell proliferation and/or inhibiting apoptosis, together with other factors including anti-apoptotic factors of the Bcl-2 family gene products, as has been reported in other tumour tissues [11]. Lelle [22] reported an increase in the proliferation index from breast benign lesion to tumour tissue. Reicher and colleagues [9] described, in tumour cell lines of squamous cell carcinomas of the head and neck, that tumour cells in G0, G1, and S phases showed a juxtanuclear localisation of IL-2, whereas tumour cells in G2 and mitosis showed an increased IL-2 expression, distributed throughout the whole cytoplasm [15]. Azzarone and colleagues [7] reported similar findings in several breast cancer cell lines, as did Reicher and colleagues [15] in squamous cell carcinomas of head and neck.

The expression of IL-2 and its receptors was higher in breast infiltrating tumours than in in situ tumour samples. This agrees with the above-mentioned role of IL-2 in tumour cell proliferation because infiltrating tumours are more aggressive than in situ tumours. In these latter, tissue keeps its glandular structure, whereas infiltrating tumour epithelial cells lose their normal structure and...
invade the adjacent stromal tissue [23]. In this context, Querzoli and colleagues [24] reported that infiltrating breast cancers have a higher proliferation index than in situ tumours.

Using immunofluorescence, Ridings and colleagues [10] found that most infiltrating ductal breast carcinomas were positive for IL-2Rβ, but only 10% were positive for IL-2Rx. In contrast, the present study of infiltrating ductal breast carcinomas revealed an intense expression of β and γ chains in 80% of cases, and of α chains in 60% of cases. Because IL-2Rβ and IL-2Rγ are necessary to form the active receptor [25], these results suggest that infiltrating breast tumours expressed functional receptors, able to respond to IL-2 stimuli.

The increased expression of the γ chain is not exclusively related to the response to IL-2 stimuli. Because this chain is also a component of receptor complexes for other interleukins, including IL-4, IL-7 and IL-9 [26,27], the function of these interleukins might also be enhanced. Several authors have demonstrated the overexpression of receptors for these interleukins in such different tumours as lung, renal, melanoma, colorectal and breast [28,29].

Several interleukins, including IL-2, IL-4, IL-5 and IL-10, have been shown to induce IL-2Rx expression in inflammatory processes and viral infections [21]. Because this chain is necessary to form the high-affinity IL-2R [30], which enhances the IL-2 signal, the increased expression of IL-2Rx in breast-infiltrating tumours suggests that the IL-2 signal is more active in this tumour group.

Conclusion

Although immunohistochemical studies cannot provide any information about the function of these molecules, the present data suggest that the development of breast tumour is associated with an increased expression of IL-2 and its three receptor chains, and this expression also seems to be associated with the malignancy of the tumour.

Competing interests

None declared.

Acknowledgements

This work was supported by grants from the Fondo de Investigaciones Sanitarias (PI020383) and the University of Alcalá.

References

1. Minami Y, Kono T, Miyazaki T, Tiguchi T: The IL-2 receptor complex: its structure, function and target genes. Annu Rev Immunol 1993, 11:245-267.
2. Whiteside TL, Herberman RB: The role of natural killer cells in immune surveillance of cancer. Curr Opin Immunol 1995, 7: 704-710.
3. Takeshita T, Aso H, Ahtani K, Ishii N, Kumaiki S, Tanaka N, Munakata H, Nakamura M, Sugamura K: Cloning the gamma chain of the human IL-2 receptor. Science 1992, 257:379-382.
4. Robb RJ, Rusk CM, Yodoi J, Greene WC: Interleukin 2 binding molecule distinct from the Tac protein: analysis of its role in formation of high affinity receptors. Immunology 1987, 64: 1987-2002.
5. Hatakeyama M, Tsudo M, Miramoto S, Kono T, Doi T, Miyata T, Miyasaka M, Taniguchi T: Interleukin 2 receptor beta chain gene: generation of three receptor forms by cloned human alpha and beta chain cDNAs. Science 1989, 244:551-556.
6. Leotta N, Kamo M, Okuma M, Ueda H, Uematsu T: The IL-2 receptor alpha-chain alters the binding of IL-2 to the beta-chain. J Immunol 1991, 147:3396-3401.
7. Azzarone B, Pottin-Clemenceau C, Krief P, Rubinstein E, Jasmin C, Scudelleri M, Indiveri F: Are interleukin-2 and interleukin-15 tumor promoting factors in human non-hematopoietic cells? Eur Cytokine Netw 1996, 7:27-36.
8. Lin WC, Yasumura S, Suminami Y, Sung MW, Nagashima S, Stans J, Whiteside TL: Constitutive production of IL-2 by human carcinoma cells, expression of IL-2 receptor, and cell growth. Immunol 1995, 155:4805-4816.
9. Reichert T, Watkins S, Stans J, Johnson JT, Whiteside TL: Endogenous IL-2 in cancer cells: a marker of cellular proliferation. J Histochem Cytochem 1998, 46:803-811.
10. Ridings J, Macaride PJ, Virad RW, Byard RW, Skinner J, Zola H: Cytokine receptor by solid tumours. Therapeut Immunol 1995, 2:57-76.
11. Royuela M, De Miguel MP, Bethencourt FR, Fraile B, Arenas MI, Paniagua R: IL-2, its receptors, and bcl-2 and bax genes in normal, hyperplastic and carcinomatous human prostate: immunohistochemical comparative analysis. Growth Factors 2000, 18:135-146.
12. Reichert T, Kashy P, Stans J, Whiteside TL: The role of endogenous interleukin-2 in proliferation of human carcinoma cell lines. Br J Cancer 1990, 61:822-831.
13. Wigginton JM, Komschlies KL, Back TC, Franco JL, Brunda MJ, Witrou RH: Administration of interleukin 12 and pulse interleukin 2 and rapid and complete eradication of murine renal carcinoma. J Natl Cancer Inst 1996, 88:38-43.
14. Wigginton JM, Park J, Grays ME, Young HA, Horcyc JK, Back TC, Brunda MJ, Stieeter RM, Ward J, Green JE, Witrou RH: Complete regression of established spontaneous mammary carcinoma and the therapeutic prevention of genetically programmed neoplastic transition by IL-12/pulse IL-2: induction of local T cell infiltration, fas/fas ligand gene expression, and mammary epithelial apoptosis. J Immunol 2001, 166:1156-1168.
15. Reichert T, Nagashima S, Kashy P, Stans J, Gao G, Dou OP, Whiteside TH: Interleukin-2 expression in human carcinomas cell lines and its role in cell cycle progression. Oncogene 2000, 19:514-525.
16. Katano M, Matsuo T, Moriski T, Naito K, Nagumo F, Kubota E, Nakamura M, Hisatsugu T, Tadano J: Increased proliferation of human breast carcinoma cell line by recombinant interleukin-2. Cancer Immunol Immunother 1994, 39:161-168.
17. Carlson G, Peterson H, Marsel M, Auger-Bourget Y, Sahraoui Y, Rubinstein E, Suarez H, Azzarone B: N-ras dependent revertant phenotype in human HT 1080 fibrosarcoma cells is associated with loss of proliferation within normal tissues and expression of adult membrane antigen phenotypes. Oncogene 1989, 4:873-880.
18. Plaisance S, Rubinstein E, Ailecherche A, Sahraoui Y, Krief P, Augery-Bourget Y, Jaomln C, Suarez H, Azzarone B: Expression of the interleukin-2 receptor in human fibroblasts and its biological significance. Int Immunol 1992, 4:739-746.
19. Sander B, Anderson J, Anderson U: Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. Immunol Rev 1991, 119:85-93.
20. Ellery JM, Nicholls PJ: Alternate signalling pathways from interleukin-2 receptor. Cytokine Growth Factor Rev 2002, 13:27-40.
21. Graffin SL, Lai SY, Xu W, Gouilleux F, Groner B, Goldsmith MA, Greene WC: Signalling through the interleukin-2 receptor β chain activates a stat-5-like DNA-binding activity. Proc Natl Acad Sci USA 1995, 92:7192-7196.
22. Lelle RJ: In situ determination of the Ki-67 growth fraction (Ki-67GF) in human tumors (studies in breast cancer). Acta Histochem Suppl 1990, 39:109-1024.
23. Tavassoli FA: Infiltrating carcinomas, common and familiar special types. In: Pathology of the Breast. Edited by Appleton and Lange. Connecticut; 1992:293-333.
24. Querzoli P, Albonico G, Ferreti S, Rinaldi R, Magri E, Nenci I: Quantitative immunoprofiles of breast cancer performed by image analysis. *Anal Quant Cytol Histol* 1999, 21:151-160.

25. Bich-Thuy LT, Bukovich M, Peffer NJ, Fauci AS, Kehrl JH, Greene WC: Direct activation of human resting T cells by IL-2: the role of an IL-2 receptor distinct from the TAC protein. *J Immunol* 1987, 139:1550-1556.

26. Russell SM, Keegan AD, Harada N, Nakamura Y, Noguchi M, Leland P, Friedman MC, Millaimea A, Puri RO, Paul WE, Leonard WJ: Interleukin-2 receptor γ chain: a functional component of the IL-4 receptor. *Science* 1993, 262:1880-1883.

27. Kondo M, Takesita T, Ishii N, Nakamura M, Watanabe, S, Arai K, Sugamura K: Sharing of interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. *Science* 1993, 262:1874-1877.

28. Cosenza L, Gorgun G, Urbano A, Foss F: Interleukin-7 receptor and activation in nonhaematopoietic neoplastic cell lines. *Cell Signal* 2002, 14:317-325.

29. Mauroer MJ, Walter W, Martin D, Zytyogel L, Elder E, Storkus W, Lotz MT: Interleukin-7 (IL-7) is produced by tissues from colorectal cancer and promotes preferential expansion of tumour infiltrating lymphocytes. *Scand J Immunol* 1997, 42:182-192.

30. Smith KA. The interleukin-2 receptor. *Annu Rev Cell Biol* 1989, 5:397-425.

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