Aromatase (CYP19) expression in tumor-infiltrating lymphocytes and blood mononuclears

Abstract Purpose: To clarify the role of lymphocytes as a possible source of estrogens. Methods: In the present study, lymphocytes were isolated from 11 surgical samples of breast cancer after tumor enzyme digestion and Ficoll/Verographine procedure. Simultaneously, using the latter procedure, mononuclears were separated from the blood of 15 female volunteers. Results: Expression of the aromatase (CYP19) gene was readily demonstrated by standard RT-PCR in blood mononuclears cultivated in the presence of 10% fetal calf serum for 48 h. In the tumor-infiltrating lymphocytes (TIL) of breast cancer patients, CYP19 expression was discovered only with the aid of nested PCR. Conclusions: The data obtained suggest that aromatase gene expression is presented in TIL at a rather low level. Nevertheless, this can have some functional significance for the estrogen-dependent growth of breast cancer tissue.

Keywords Aromatase expression · Tumor-infiltrating lymphocytes · Mononuclears · Breast cancer

Introduction

Conversion of androgens to estrogens is catalyzed by the enzyme aromatase that belongs to the XIX class of cytochrome P450 (CYP19). The ability to be a source of extragonadal estrogen synthesis is characteristic of different normal tissues as well as for certain malignant tumors, breast cancer included (Santen et al. 1994, 1997; Miller 1996; Dowsett et al. 1996; Reed and Purohit 1997). The cellular substrate participating in this process in breast cancer tissue is rather heterogeneous. Earlier – in collaboration with the group of Dr. R. Santen – we proposed the so-called “three-component model” of estrogen formation in breast tumors. This model postulated that besides epithelial (Esteban et al. 1992; Berstein et al. 1996) and stromal (Santen et al. 1994, 1997) cells, the total estrogenic tumor pool has input from the cells of lymphocytic-macrophagel infiltrate (Berstein et al. 1995). While the role of macrophages as a source of extragonadal estrogen production is demonstrated rather convincingly (Mor et al. 1998), the clear evidence of this in relation to lymphocytes and – specifically – to tumor-infiltrating lymphocytes (TIL) has not been presented. The proportion of lymphocytes in the mass of breast tumor cells may vary from 3–5% to more than 40%. Later investigations have increasingly shown the “U-shaped” character of dependence between lymphocytic tumor infiltration and survival rate of breast cancer patients (Marsigliante et al. 1999). Worsening results of treatment, along with pronounced lymphocytic infiltration of tumor tissue may be associated with the ability of these cells to be not only immunocytes, but also hormonocytes, i.e., to participate in hormone secretion (Besedowsky and DelRey 1996). If lymphocytes really possess cellular machinery to produce estrogen this could be very important in explaining how the same lymphocytic infiltrate can provide such different signals for breast tumor growth (estrogens) and its blockade (cellular immunity).

Previously we obtained data on the ability of both blood mononuclears (mainly lymphocytes) from heal-
thry women and breast cancer patients and tumor-infiltrating lymphocytes from breast tumors to convert androstanediol as evaluated by tritiated-water release, and suggested that more direct confirmation of the presence of aromatase in lymphocytes (including TIL) can be obtained by the use of molecular-genetic methods of investigation (Berstein et al. 1998). The aim of the current work was to study aromatase (CYP19) gene expression in blood mononuclears and in cells isolated from breast cancer lymphoid infiltrates.

**Material and methods**

Samples of cubital vein blood for this study were received after night fasting from 15 females with ages from 23 years to 57 years; 12 of them were postmenopausal. Plasma was applied on Ficoll/Verographine (d = 1.077) and the fraction of mononuclears was isolated. More than 90% of cells were alive and presented as lymphocytes. Part of the samples (non-cultivated cells) went directly through a total RNA isolation procedure (see below) while another part was cultivated for 48 h at 37 °C in RPMI-1640 containing 10% fetal calf serum, penicillin, and streptomycin at 37 °C for 48 h. Tumor tissue obtained from 11 breast-cancer patients (aged from 42 years to 72 years; nine patients were among the 15 studied samples of blood cells, seven were not cultivated before total RNA isolation and another eight samples were cultivated in RPMI-1640 containing 10% fetal calf serum for 48 h. A statistically significant difference between these two types of samples was obtained when evaluating CYP19 gene expression. Namely, this expression was positive only in one of seven non-cultivated samples of lymphocytes, while it was strongly positive in six of eight cDNA probes resulting from cells subjected to cultivation (P = 0.02, chi-square method). Examples of data are demonstrated in Fig. 1a. In all ten samples of tumor-infiltrating lymphocytes the expression of the aromatase gene could not be discovered when only standard (1st round) PCR was performed, whether these cells were cultivated before isolation of RNA or not. In contrast to this, all six non-cultivated TIL samples that went through the nested PCR (2nd round) procedure gave a remarkable positive response and reaction products appeared as a major band of the expected size (see Fig. 1b).

**Discussion**

Breast tumors consist of different cell types: in addition to malignant epithelial cells, there are stromal cells of fibroblastic origin, and mononuclear cells, mainly lymphocytes and macrophages. Immunocytochemical investigations have shown that aromatase can be detected both in epithelial and in stromal tumor cells, as well as in macrophages (see Introduction), but its presence in cells of the lymphocytic infiltrate or in blood lymphocytes could not be convincingly demonstrated or rejected by this method, due to technical difficulties (Berstein et al. 1993, 1996, 1998). In the present paper, expression of the aromatase (CYP19) gene was evaluated by polymerase chain reaction. In blood lymphocytes positive results were achieved more frequently if these cells were cultivated in RPMI-1640 containing 10% fetal calf serum (FCS) for 48 h (Fig. 1a). In tumor-infiltrating lymphocytes, CYP19 expression could be discovered only with the more sensitive nested PCR (Fig. 1b), and – in contrast to blood lymphocytes – the cultivation of cells before RNA isolation led to the disappearance of the expected signal. These data demonstrate the presence of mRNA transcripts for aromatase in lymphocytes, though as one can see, special conditions should be used to discover CYP19 expression in these cells.

Existing data indicate that tumor lymphocytic infiltration results mostly from the lymphocytes chemotactically attracted from the blood, and the difference revealed in the ability of blood and tumor lymphocytes to express aromatase deserves explanation. Although blood lym-