Background: The production of the cytokine interferon gamma (IFN \( \gamma \)) by activated peripheral blood mononuclear cells may be reduced in patients with invasive cervical carcinoma. This study was designed to assess the prognostic value of intratumoral IFN \( \gamma \) messenger RNA (mRNA) levels in such patients. Methods: Biopsy specimens of primary cervical lesions were obtained from 27 patients with invasive squamous cell carcinoma before they received any therapy. Two prognostic groups were considered: 1) a group of 14 patients who had no apparent disease recurrence and who were alive 2 years after diagnosis (good-prognosis group) and 2) a group of 13 patients who had disease recurrence or died during the 2-year follow-up (poor-prognosis group). A competitive reverse transcription–polymerase chain reaction (RT-PCR) assay was used to measure levels of IFN \( \gamma \) and \( \beta \) actin mRNA. The expression of human leukocyte antigen (HLA) class II proteins (which is stimulated by IFN \( \gamma \)) in tumor cells was studied by immunostaining. Results: Tumor specimens from all 14 patients in the good-prognosis group contained more than \( 10^3 \) IFN \( \gamma \) mRNA copies per 5 \( \times \) \( 10^5 \) \( \beta \) actin mRNA copies, whereas tumor specimens from only six of the 13 patients in the poor-prognosis group contained this level of IFN \( \gamma \) mRNA (two-sided \( P = .006 \)). No clear relationship was observed between levels of IFN \( \gamma \) mRNA and T-cell or natural killer cell infiltration in tumors; however, a statistically significant association was observed between HLA class II expression on tumor cells and IFN \( \gamma \) mRNA levels (two-sided \( P = .01 \)). Conclusions: A subgroup of poor-prognosis cervical carcinoma patients who have low levels of intratumoral IFN \( \gamma \) mRNA was identified. [J Natl Cancer Inst 1998; 90:287–94]

Human papillomavirus (HPV) DNA is detected in more than 90% of all uterine cervical tumors (1), and antigenic epitopes derived from HPV early-region genes 6 and 7 (E6 and E7) are expressed and presented by tumor cells (2). Cell-mediated immune response are believed to be essential for control of the growth of HPV-associated tumors; the growth of these tumors has been prevented by induction of anti-HPV effector T cells in animal models (3). In addition, adoptive transfer of a cytotoxic T lymphocyte (CTL) clone directed against an E7 peptide was able to eradicate established tumors associated with HPV type 16 infection (4). In humans, an increased incidence of HPV-associated cervical lesions has been observed in immunosuppressed individuals with defective cell-mediated immunity (5). The generation of CTLs against the E6 and E7 epitopes seems difficult, since in a clinical trial using recombinant vaccinia virus vector expressing HPV E6 and E7 complementary DNA (cDNA), CTLs were detected in only one of eight cervical cancer patients and the observed CTL activity was very transient (6). After in vitro stimulation, a T-cell response against an HPV E7 peptide was found in only two of 11 cervical carcinoma patients, whereas, in the same experiment, CTL immunity against an influenza virus epitope was evident in eight of the 11 patients (7). However, because the protocol used in this study detected virus-specific memory T cells, the poor natural CTL immunity against HPV may be related to immunosuppression at the tumor site.

Dysregulation of the expression of a human leukocyte antigen (HLA) molecule on cervical tumor cells has been demonstrated, with frequent down-regulation of HLA class I molecules and aberrant expression of HLA class II antigens by tumor cells (8,9). These changes modify the antigen presentation properties of tumor cells and could represent escape mechanisms from immune rejection.

Interferon gamma (IFN \( \gamma \)) is a cytokine produced by activated T cells and natural killer (NK) cells that enhances cellular immune responses by increasing T-cell cytotoxicity and NK cell activity. By increasing the rate of transcription of the HLA gene, it up-regulates the expression of HLA class I and II molecules in both normal and tumor cells (10,11). The striking correspondence between the various biologic activities of IFN \( \gamma \) and the immunologic modifications observed in cervical carcinomas prompted us to measure IFN \( \gamma \) messenger RNA (mRNA) at the tumor site by means of a quantitative polymerase chain reaction (PCR) assay. An association between the level of IFN \( \gamma \) gene expression on tumor and the observed CTL activity was very transient (6).

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expression and HLA class II expression by cervical tumor cells in vivo was also investigated.

Materials and Methods

Patients and Tissues

Biopsy specimens were obtained from the primary cervical lesions of 27 patients with invasive squamous cell carcinoma before they received any therapy. Written informed consent was obtained from each subject according to institutional rules. Tissues were divided into two equal parts: One portion was fixed in formalin for histologic analysis, and the other was snap-frozen in liquid nitrogen and stored at −70 °C for RNA extraction. This protocol was approved by a regional ethics committee.

Clinical staging was performed according to the classification of the International Federation of Gynecology and Obstetrics (FIGO) (12). Stages Ia and Ib were regrouped into class I (17 case subjects), stages IIA, IIB, and IV into class III (two case subjects). Age at diagnosis ranged from 28 to 77 years. Two prognostic groups were considered: 1) a group of 14 patients who had no apparent disease recurrence and who were alive 2 years after diagnosis (good-prognosis group) and 2) a group of 13 patients who had disease recurrence or died during the 2 years following diagnosis (poor-prognosis group).

Cell Line and Culture of Tumor-Infiltrating Lymphocytes

The established HeLa cervical tumor cell line was obtained from the American Type Culture Collection, Rockville, MD. It was grown in RPMI-1640 medium (BioWhittaker, Inc., Walkersville, MD) supplemented with 10% fetal calf serum and 100 U/mL penicillin–50 μg/mL streptomycin, 5% sodium pyruvate, and 0.01% mercaptoethanol (all from Sigma Chemical Co., St. Louis, MO).

Tumor-infiltrating lymphocytes were derived from tumors. They were cultured after enzymatic digestion as previously described (13).

Immunocytochemistry

Immunohistochemical staining was performed by use of the universal rabbit–mouse labeled streptavidin–biotin method (Dako LSAB kit; Dako Corp., Santa Barbara, CA). The tumor immunologic infiltrates were characterized with the use of antibodies to T cells (anti-CD3 rabbit polyclonal antibody at a 1:100 phosphate-buffered saline [PBS] dilution; Dako Corp.) and NK cells (anti-NK1 mouse monoclonal antibody at a 1:50 PBS dilution; Dako Corp.). We evaluated cell infiltration by counting random high-power fields with an American Optical microscope using a 45x objective with a 0.47-mm-diameter field.

We studied the HLA class II expression by immunostaining tissue with the mouse CR3/43 monoclonal antibody. This antibody recognizes the HLA-DP, -DQ, and DR antigens (Dako Corp.).

As previously reported, expression of major histocompatibility complex II was considered positive when 25% or more of tumor cells showed positive staining (14). Histologic interpretations were made in a blinded manner by two independent observers.

Quantification of mRNA

The levels of specific mRNAs were measured as previously described (15). Briefly, quantitative titration of β actin and IFN γ mRNA was performed by competitive reverse transcription–PCR run to saturation, which always included an internal DNA standard for IFN γ or β actin for each reaction. Internal DNA standards for IFN γ and β actin were obtained from the PCR products, respectively, generated with IFN γ primers (sense, positions 178–199; antisense, positions 495–517) and β actin primers (sense, positions 67–92; antisense, positions 729–754). The sequences of IFN γ and β actin PCR oligonucleotide primers and runoff (RO) oligonucleotide primers are as follows: IFN γ 5′-GGTTCCTCTGCTGTATGGCC-3′, IFN γ 3′-GTTGGGACATTCAAGTC-5′, and IFN γ RO (TTGAAGTAAAGGAGACATTTGGCCT); β actin 5′-TCCTGACACAGGCGTCCTC-3′, β actin RO (TTGAAGTAAAGGAGACATTTGGCCT), and β actin RO (TTGAAGTAAAGGAGACATTTGGCCT).

A deletion of a 4-base-pair fragment from wild-type cDNA was introduced on β actin standard at positions 192–196, and an insertion of 4 base pairs was added to IFN γ internal standard at positions 231–235. Amplification reactions were performed in a 50-μL mixture containing 50 U/mL of Taq polymerase (Promega Corp., Madison, WI), 200 μM (each of the four) deoxynucleotide triphosphates (dNTPs), 0.2 μM of each of the two primers, and 1.5 mM MgCl₂ in a standard PCR buffer (Promega Corp.). The reaction mixtures were overlaid with 50 μL of mineral oil. The reaction was performed in a thermal cycler machine (Perkin-Elmer Cetus, Norwalk, CT) for 40 cycles of 1 minute at 94 °C, 1 minute at 55 °C, and 1 minute 30 seconds at 72 °C, followed by 10 minutes at 72 °C. A 2-μL aliquot of the amplified solution was then mixed in a final reaction volume of 10 μL containing 0.1 μM fluorescent primer, 20 U/mL of Taq polymerase, 200 μM (each of the four) dNTPs, and 3 mM MgCl₂ in Promega buffer and submitted to one PCR cycle under the same conditions described above (RO reaction to label PCR products). The RO reaction products were mixed with an equal volume of a 20 μM EDTA–95% formamide solution and heat denatured at 80 °C for 10 minutes, and a 2-μL aliquot of the resulting mixture was loaded on a 6% acrylamide–8M urea gel and subjected to electrophoresis for 4 hours on an automated DNA sequence analysis machine (373 A DNA sequence; PE Applied Biosystem, Foster City, CA). A computer program was developed by C. Panetier (Institut Pasteur, Paris, France) to measure the length and area for each detected peak (15). The peak area ratio between known concentrations of standard DNA and target cDNA enabled us to determine the concentration of target cDNA derived from the mRNA to be quantified.

We took a number of precautions to avoid PCR artifacts, including the use of aerosol-resistant pipette tips, aliquoted reagents, and pipettes dedicated for PCR use and the assembly of reactions in laminar flow hoods. Negative control reactions were performed for every experiment and included all reagents necessary for PCR, however, instead of receiving reverse-transcribed RNA (cDNA), the reaction mixtures received buffer alone or aliquots of non-reverse-transcribed RNA.

Because of insufficient material in some biopsy specimens, we were able to measure IL6 mRNA in only 26 samples.

Statistical Analysis

Comparisons between IFN γ or IL6 mRNA levels and different cervical cancer patient groups were assessed by use of the two-sided Mann–Whitney U test. Relationships between IFN γ gene expression and prognostic groups were analyzed by use of the two-sided χ² test with Yates correction when necessary. A two-sided Spearman’s test was used to measure correlations between immune cell infiltration and IFN γ mRNA levels. Bivariate analyses were performed by use of the Mantel–Haenszel test. A P value of less than .05 was considered to be significant.

Results

Association Between IFN γ mRNA Levels and Disease-Free Survival in Cervical Carcinoma

We report a statistically significant association between IFN γ mRNA levels assessed by quantitative PCR in biopsy specimens derived from patients with invasive cervical carcinoma and survival. Indeed, all patients (14 of 14) in the good-prognosis group expressed more than 10³ IFN γ mRNA copies per 5 × 10³ β actin mRNA copies, whereas fewer than 1000 IFN γ mRNA copies per 5 × 10³ β actin mRNA copies were detected in seven of 13 patients in the poor-prognosis group (P = .006) (Table 1). The median value for the ratio of IFN γ mRNA copies to 5 × 10³ β actin copies in the good-prognosis group was 87 500 compared with 500 in the poor-prognosis group (Fig. 1, A). We did not find any association between IFN γ mRNA levels and FIGO status. Indeed, low IFN γ mRNA copy numbers were observed in four of 17 patients in the FIGO stage 1a or 1b group and in three of 10 patients in the other stage group (P> .9). Furthermore, when adjusted for FIGO classification, IFN γ status remained an independent prognostic factor (P = .0085).

We have previously reported (15) an increase in IL6 mRNA levels in invasive cervical carcinoma as compared with cervical intraepithelial neoplasia and normal cervix. In this study of pa-
patients with invasive cervical carcinoma, although the median of IL6 mRNA levels was higher in the poor-prognosis group than in the good-prognosis group (50,000 mRNA copies per 5 × 10^5 β actin mRNA copies versus 600 mRNA copies per 5 × 10^5 β actin mRNA copies), the difference was not statistically significant (P < 0.29) (Fig. 1, B).

**Correlation Between CD3 T-cell and NK Cell Infiltrates and IFN-γ mRNA Expression**

Since T cells and NK cells are the major cells producing IFN-γ in vivo, we attempted to correlate IFN-γ mRNA expression to CD3-positive T cells and CD57-positive NK cells. As shown in Fig. 2, we could not demonstrate any clear association between IFN-γ mRNA levels and T lymphocytes (Fig. 2, A) or NK cell infiltration (Fig. 2, B). IFN-γ mRNA determination and immune cell infiltration evaluation were performed on the same biopsy specimens for each patient. Moreover, assessment of CD3 infiltration by mRNA quantitation also did not show any significant relationship with IFN-γ gene expression (data not shown).

In accordance with these results, although T-cell infiltration and NK cell infiltration were higher in the good-prognosis group (median CD3: 48 per field; median NK cells: nine per field) than in the poor-prognosis group (median CD3: 30 per field; median NK cells: six per field), the difference did not reach statistical significance (P > 0.1).

**In Vitro Correlation Between IFN-γ mRNA Expression and Activated T Cells**

Because of the absence of a relationship between IFN-γ mRNA levels and CD3 T-cell infiltration in vivo, we attempted to control these results in vitro. We mixed different concentrations of purified IL2 (interleukin-2)-activated T cells with the HeLa cervical carcinoma cell line. The total number of cells always remained constant at a total of 2 × 10^7. Quantitation of β-actin mRNA therefore allowed us to normalize IFN-γ copy

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**Table 1.** Relationships between level of interferon gamma (IFN-γ) gene expression and survival in patients with invasive cervical carcinoma

<table>
<thead>
<tr>
<th>Group</th>
<th>&gt;1000 copies</th>
<th>≤1000 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good-prognosis, cervical cancer patients with favorable outcome</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Poor-prognosis, cervical cancer patients with recurrence or poor survival (&lt;24 mo)</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

*All IFN-γ messenger RNA (mRNA) quantitations were normalized per 5 × 10^5 β-actin mRNA copies. P = .006 (χ² two-sided test with Yates corrections).*

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**Fig. 1.** A) Interferon gamma (IFN-γ) gene expression is associated with clinical outcome in patients with invasive cervical carcinomas. Twenty-seven complementary DNA (cDNA) preparations derived from messenger RNA (mRNA) extracted from cervical tumor biopsy specimens were coamplified with internal standard for IFN-γ or β-actin with specific IFN-γ or β-actin primers. A runoff reaction with a third nested specific fluorescent IFN-γ or β-actin primer was then performed. After electrophoresis, the fluorescent profile was recorded, and peak areas were computed. IFN-γ gene expression was compared between two clinical prognostic groups. B) Interleukin 6 (IL6) gene expression in patients with invasive cervical carcinoma. Twenty-six cDNA preparations derived from mRNA extracted from cervical tumor biopsy specimens were coamplified with internal standard for IL6 or β-actin with specific IL6 or β-actin primers. A runoff reaction with a third nested specific fluorescent IL6 or β-actin primer was then performed. After electrophoresis, the fluorescent profile was recorded, and peak areas were computed. IL6 gene expression was compared between two clinical prognostic groups.

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numbers. We demonstrated a linear relationship between the number of activated T cells and IFN $\gamma$ mRNA levels (Fig. 3). The variation between expected IFN $\gamma$ mRNA values extrapolated from the first results obtained with $5 \times 10^5$ T cells in the preparation and the measured IFN $\gamma$ mRNA expression never exceeded 7%. This result confirms the reliability and linearity of our methodology and indicates that the presence of cervical carcinoma cells should not interfere with the determination of IFN $\gamma$ mRNA copies. We also showed that the interval of linearity of the quantitative PCR assay for IFN $\gamma$ mRNA measurement ranged between $10^2$ and $10^7$ mRNA copies per $5 \times 10^5$ $\beta$ actin mRNA copies.

### Relationship Between IFN $\gamma$ Gene Expression and HLA Class II Antigen Expression on Tumor Cells

In vitro, cervical cell lines do not constitutively express HLA class II antigens, but IFN $\gamma$ may induce them to do so (16). In vivo, in contrast to keratinocytes that do not normally express HLA class II antigens, cervical tumor cells often exhibit positive immunostaining for these molecules (17). In our analysis, a statistically significant expression of HLA class II on cervical tumor cells was observed in 12 of 24 sections tested (Figs. 4 and 5). In all tissue sections, variable numbers of class II positive cells infiltrating stromal tissue were present, which aids in the control of the quality of tissue preparation for immunocytochemistry (Fig. 4). These cells may correspond to professional (i.e., committed) antigen-presenting cells (e.g., macrophages or
dendritic cells) that constitutively express HLA class II. As shown in Fig. 5, IFNγ gene expression and HLA class II expression on tumor cells were clearly related. In the group of tumors expressing HLA class II, the median IFNγ mRNA level was 85,000 mRNA copies per $5 \times 10^5$ β actin mRNA copies; in contrast, in the absence of class II expression by tumor cells, IFNγ mRNA reached only a median value of 875 mRNA copies per $5 \times 10^5$ β actin mRNA copies ($P = .01$). It is noteworthy that this correlation was particularly marked for extreme values of IFNγ mRNA levels. For example, HLA class II expression on tumor cells was absent in six of seven case subjects when IFNγ mRNA levels were lower than 1000 copies per $5 \times 10^5$ β actin mRNA copies, whereas its up-regulation was associated with IFNγ mRNA levels higher than 100,000 copies per $5 \times 10^5$ β actin mRNA copies in six of seven case patients (Fig. 5). We did not find any association between disease stage and HLA class II status. Indeed, six of 14 patients belonging to the FIGO stage T1a and T1b group expressed HLA class II on tumor cells; in contrast, in the T2–T3–T4 group of patients, this expression was observed in six of 10 patients ($P = .4$).

**Discussion**

We report an association between intratumoral IFNγ mRNA levels (as assessed by a quantitative PCR assay) and clinical outcome in patients with primary invasive cervical carcinoma. Cervical cancer patients who exhibited fewer than 1000 IFNγ mRNA copies per $5 \times 10^5$ β actin mRNA copies were identified only in the population with high risk of recurrence or low survival. However, six of 13 patients belonging to this poor-prognosis group also expressed high levels of IFNγ mRNA. Several studies (18–20) have indicated a switch in the pattern of cytokine from TH1 (IL2, IFNγ) to TH2 (IL4, IL5, IL10) groups in cancer patients compared with healthy control subjects, but the clinical prognostic value of this finding has not been determined. A decrease in IFNγ production by activated peripheral blood mononuclear cells derived from cervical cancer patients has been previously demonstrated, but its significance has not been established (21). Dysregulation of IFNγ levels occurs early in the course of the disease. Indeed, Pao et al. (22) showed that IFNγ mRNA levels were significantly reduced in cervical intraepithelial neoplasia and cervical cancer tissue compared with normal cervix. Moreover, the level of IFNγ production by phytohemagglutinin-stimulated peripheral blood mononuclear cells was reported to be lower when the cells were from women with HPV genital infections than when they were from control subjects (23). A defect in IFNγ expression at the tumor site may favor tumor progression by various mechanisms. In vitro, IFNγ was found to repress the expression of HPV type 16 and 18 genes in immortalized cell lines and to inhibit the growth of most cervical carcinoma cell lines (24). IFNγ was observed to change the structure and function of proteasome subunits and to enhance peptide presentation to cytotoxic T cells (25). In addition, it was demonstrated that some tumor cells defective in specific presentation pathways could recover this function after treatment with IFNγ (26).

![Fig. 4. Immunostaining of invasive carcinoma with monoclonal anti-human leukocyte antigen (HLA) class II antibody. Positive (A) and negative (B) HLA class II expression on cervical tumor cells. In all sections, varying numbers of positive cells infiltrating stroma tissue were present, which confirms the quality of tissue preparation.](image1)

![Fig. 5. Association between interferon gamma (IFNγ) gene expression and human leukocyte antigen (HLA) class II immunostaining on cervical tumor cells. HLA class II expression was considered positive when 25% or more tumor cells showed positive staining. mRNA = messenger RNA.](image2)
An increase in IL6 gene expression in tumor tissue as compared with normal epithelium or dysplasia has now been established by various research groups, including our own (15,27,28). In this series, although higher IL6 mRNA concentrations were recorded in the poor-prognosis cervical cancer patient group than in the good-prognosis group, these results were not statistically significant. In mice, when IL6-transfected tumors were compared with wild-type tumors, the outcome was often unpredictable and always depended on the phenotype of the parental tumor (29,30).

Surprisingly, we did not find any correlations between IFN γ mRNA levels and T-cell or NK tumor cell infiltration. To support these in vivo data, we reconstructed an in vitro model, in which different numbers of activated T cells and cervical tumor cells were mixed. A linear relationship was demonstrated between IFN γ gene expression and the number of stimulated T cells present in culture (Fig. 3). Because IFN γ mRNA measurement and tumor cell infiltration determinations were performed by different techniques, a bias could have been introduced, although slides for immunocytochemistry and RNA used for PCR were derived from the same frozen tissue. For some samples, T-cell infiltration was also determined by quantitative PCR, which confirmed the immunocytochemistry results (data not shown). In accordance with these results, other groups (31,32) showed that tumor-infiltrating lymphocytes derived from breast or ovarian carcinoma often express very low levels of IFN γ. This observation could account for a state of anergy of tumor-infiltrating lymphocytes that often exhibit impaired cytotoxic functions and failure to proliferate (13,33).

An increased number of CD8-positive T subsets has been reported in invasive cervical carcinoma with a decrease in the ratio of CD4 to CD8 (34,35). The recent identification of CD8 T cells lacking cytotoxic functions that did not produce IFN γ but developed the capacity to produce TH2 cytokines (IL4, IL5, IL10) recalls previous data about a "suppressor role" for these CD8 T cells infiltrating invasive cervical carcinoma (36,37).

Aberrant expression of HLA class II molecules has been demonstrated in cervical tumors (9,17). Its direct relationship to the malignant transformation process, or rather its up-regulation by molecules present in the tumor microenvironment, is debated. A clear association was shown between IFN γ mRNA levels at the tumor site and expression of class II molecules by tumor cells (Fig. 5). Six of seven patients with low IFN γ mRNA levels did not express HLA class II on tumor cells. Various groups (16,38) have shown that cervical carcinoma cell lines grown in vitro do not express constitutive HLA class II molecules; however, they can be induced at both the mRNA and membrane protein levels by IFN γ in a dose-dependent way. Therefore, in vivo, the level of IFN γ could be related to HLA class II expression on tumor cells. It is well known that cytokines such as IL4 or TNFα (i.e., tumor necrosis factor α) up-regulate IFN γ-induced HLA class II expression, whereas other cytokines such as TGFβ1 (i.e., transforming growth factor β1), IFN α, IFN β, and IL10 down-regulate this effect (11). This situation may explain discrepancies between IFN γ mRNA levels and HLA class II expression in some individual cases. In a recent study, using nonquantitative PCR, Matsushita et al. (39) reported an association between IFN γ mRNA detection and HLA DR expression in normal epithelia adjacent to colorectal carcinoma.

The absence of IFN γ mRNA is often observed in parallel with negative immunostaining for HLA DR expression both in normal epithelia and in colorectal cancer cells, which is in accordance with our results. However, IFN γ detection was not associated with HLA DR expression on tumor cells, which contrasts with our study. This relative discrepancy could be explained by the quantitative versus nonquantitative methods used to measure mRNA and the focus on HLA DR expression in the previous study (39) compared with HLA DR, DP, and DQ expression in the present analysis.

The significance of HLA class II expression on tumor cells may be complex. Indeed, various mouse tumors have been transfected with syngeneic HLA class II genes, and the resulting transfectants were very effective vaccines against subsequent challenges with wild-type class II-negative tumors (40). However, it was recently shown that, in this model of class II transfected tumors, generation of tumor immunity requires in vivo induction of tumor cell-encoded B7, a costimulatory molecule recognized by T cells (41). The absence of B7 molecules on tumor cells expressing HLA class II molecules may lead to T-cell anergy and may contribute to the escape of tumors from destruction by the host’s system (42). We did not investigate B7 expression in invasive cervical carcinoma, but the finding of B7 molecule expression only in spontaneously regressing melanoma and not in other established solid tumors reinforces its role in induction of antitumor immunity (43).

A down-regulation of HLA class I antigen with frequent allele-specific alteration was demonstrated in cervical carcinoma (8,9,14). Technical difficulties and insufficient material did not allow us to confirm these previous results or correlate this defect with IFN γ mRNA level. However, cervical carcinoma with HLA class I down-regulation showed abundant MHC heavy-chain transcripts in carcinoma cells (44). This finding indicates that mechanisms responsible for this dysregulation occur at the posttranscriptional level, whereas IFN γ regulates MHC class I expression mainly at the transcriptional level (11).

Since the decrease in IFN γ mRNA expression in a group of cervical cancer patients may not be simply related to the number of infiltrating T or NK cells (Fig. 2), other factors could also play a role in this phenomenon. It was recently shown that a decrease in IL12 production by macrophages caused by tumor-derived factors such as prostaglandin E2 or phosphatidyl serine resulted in impaired IFN γ production in mammary tumor-bearing mice (45). An increase in prostaglandin E2 secreted by peripheral blood mononuclear cells has also been reported during the progression of cervical carcinoma (21).

HPV did not seem to play a role in IFN γ gene expression, since we did not find any association between HPV-associated tumors and IFN γ mRNA levels (data not shown). Cromme et al. (46) also reported an absence of an association between the presence of specific HPV genotypes and HLA expression pattern in cervical carcinomas.

In conclusion, we propose that IFN γ gene expression may represent a marker of activated T cells or NK cells that could be more relevant as a prognostic indicator in cervical cancer than total infiltrating immune cells, which include anergic cells. Since low IFN γ production at the tumor site may be directly involved in the poor outcome of cervical cancer patients, therapeutic strategies designed to reverse this defect may be consid-
ered. Preliminary reports (47, 48) about the partial efficacy of IFN γ in the treatment of cervical intraepithelial neoplasia and overt cervical squamous cell carcinoma support this hypothesis.

Furthermore, during the follow-up of cervical cancer patients enrolled in various clinical trials of vaccination against HPV-derived proteins, determination of IFN γ mRNA level and HLA class II expression at the tumor site may help to better analyze immunological and clinical responses.

References


(40) Chen PW, Ananthaswamy HN. Rejection of K1735 murine melanoma in syngeneic hosts requires expression of MHC class I antigens and either class II antigens or IL-2. J Immunol 1993;151:244–55.


Notes

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