Interferon γ and Tumor Necrosis Factor α mRNA Expression in Mycosis Fungoides Progression

To the Editor:

In a recent issue of Blood, Sarris et al. suggested that cytokine loops involving interferon-γ (IFNγ) and the IFNγ-inducible protein (IP-10) may explain the epidermotropism of cutaneous T-cell lymphoma (CTCL). The basis of this hypothesis has been the overexpression of IP-10 shown in skin biopsies from patients with mycosis fungoides (MF) and a review of the literature, indicating cutaneous IFNγ mRNA expression in MF patients.

In fact, there are reports from Saed et al. and Vowels et al. showing the presence of IFNγ in the skin of these patients. However, these reports alone could not sufficiently support the hypothesis from Sarris et al., because IFNγ was also detected by these groups in healthy skin (not showing epidermotropism).1,2 The limitation of the IFNγ gene expression studies in MF performed before is the use of reverse transcriptase-polymerase chain reaction (RT-PCR) methods giving only qualitative but not quantitative information about gene expression. Therefore, a clear evidence for IFNγ mRNA overexpression in MF failed hitherto.

Very recently, we were able to show that progression of MF is associated with increasing overexpression of IL-10 mRNA using a semi-quantitative RT-PCR (Asadullah et al., manuscript submitted). Because it has been shown that IL-10 suppresses the production of IFNγ, we speculated a decreasing IFNγ mRNA level along with MF progression.

In fact, a stage-dependent decrease of IFNγ mRNA expression was observed (Table 1). IFNγ mRNA was detected more frequently in patch stage (7 of 11 cases) compared with the advanced stages (plaque and tumor stage, 2/9) of MF (P < .05, χ² assay). Only in patch stage MF, but not in the more advanced stages, IFNγ mRNA was detected with significantly higher frequency than in healthy skin (P < .01, χ² assay).

Because a lack of IFNγ overexpression would be contrary to the hypothesis that this cytokine induces IP-10 in MF, we further investigated the expression of tumor necrosis factor α (TNFα), which also has been shown to induce IP-10.3 The mean value (± SD) of TNFα expression of all skin samples from MF patients (230 ± 274) was higher compared with healthy skin (122 ± 127, P > .05, Mann-Whitney test). No significant differences were shown by analyzing the frequency and the level of TNFα mRNA expression in different MF stage lesions and healthy skin (Table 1).

Failing to detect IFNγ in healthy skin indicates a relatively low sensitivity of our test system, because other groups detected IFNγ mRNA in higher but different frequencies.1,2 However, because enhanced IFNγ mRNA expression was shown for patch stage but not for advanced stages of MF in our study, a decrease of IFNγ expression in the course of progression is very likely and a significant overexpression of this cytokine could be excluded for advanced tumor stages.

We do not believe that our results are completely contrary to the hypothesis that IFNγ/IP-10 expression may explain the epidermotropism in MF. In fact, on one hand, our data may support this hypothesis because (1) we could show IFNγ overexpression in the early stages of MF, when significant epidermotropism is detectable; and (2) the lack of IFNγ detection in advanced MF stages would explain our former observation that progression of the disease into tumor stage often results in a loss of epidermotropism.5

However, on the other hand, Sarris et al. detected IP-10 also in advanced MF stages, for which we excluded high IFNγ expression in our samples. This would suggest that other factors, such as TNFs, which shows a tendency for higher expression throughout MF progression, may be responsible for induction of IP-10, at least in advanced stages.

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REFERENCES


Table 1. IFNγ and TNFα mRNA Expression in Skin Samples (Incidence of Detection, Mean of Arbitrary Units ± SD)

<table>
<thead>
<tr>
<th>IFNγ</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of Detection</td>
<td>Mean ± SD (arbitrary units)</td>
</tr>
<tr>
<td>MF</td>
<td></td>
</tr>
<tr>
<td>Patch stage</td>
<td>7/11*</td>
</tr>
<tr>
<td>Plaque stage</td>
<td>2/6</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>0/3</td>
</tr>
<tr>
<td>Healthy skin</td>
<td>0/8</td>
</tr>
</tbody>
</table>

* P < .01 compared with healthy skin (χ² assay).
Response: Cytokines and IP-10 in CTCL

Recently, we proposed that the epidermotropism of CTCL could be explained by cytokine loops involving secretion of the chemotactic cytokine IP-10 by keratinocytes and of IFNγ by the lymphoid infiltrate.1 We based the model on previous reports of high frequency of IFNγ mRNA in the epidermis of CTCL lesions,3 on our own immunohistochemical detection of IP-10, and preliminary immunohistochemical data confirming the presence of IFNγ in some CTCL lesions. We concluded that "additional work is needed to fully define the identity and the source of IP-10 inducer."4 In our report, we also proposed interleukin-1α as a possible inducer of IP-10 in CTCL on the basis of published work.5,6 Because TNFα is itself an inducer of IP-10 and has synergistic effects in the presence of IFNγ,5,6 it is logical to determine if TNF-α is present in CTCL lesions.6

Asadullah et al used an unspecified (7 semiquantitative) PCR method to determine the expression of TNFa and IFNγ mRNA in skin biopsies of patients with CTCL. They present their data, nevertheless, in a quantitative manner. From their data, we calculated the frequency of cytokine expression and the corresponding 95% confidence intervals (CI) and show them in Table 1. The investigators concluded that IFNγ mRNA was detected only in patch stage CTCL with higher frequency than in normal skin and that TNF-α may contribute to the epidermotropism of CTCL.

Several points should be considered:

1. It is probably not appropriate to use semiquantitative PCR and then present data in the quantitative manner as is implicit in the calculation of standard deviation. It is also somewhat misleading to present "arbitrary units" for TNFa and IFNγ mRNA levels, because it is not clear that the IFNγ PCR units are equivalent to the PCR units of TNFα. The biologically important molecules are the proteins and not the mRNAs. Because IFNγ and TNFα show marked synergy for IP-10 induction in vitro, the PCR results do not indicate the relative amounts of IP-10 TNFα or IFNγ secreted by lesional keratinocytes in vivo.

2. Because of the small number of patients studied, the 95% CI are wide, and there is probably no statistically significant difference between the frequency of IFNγ expression among the various stages of CTCL (Table 1). Analysis of more patients is needed for these differences to be statistically valid and biologically meaningful. For instance, Nicholoff et al7 detected IFNγ mRNA in 100% of 7 patients with CTCL, but the corresponding intervals were 95% CI of 59% to 100%.

3. Because small amounts of IP-10 are present in the basal keratinocyte layer of normal epidermis,8 it should not be surprising that inducers such as IFNγ or TNFα are also present there. This does not detract from our model that was specifically proposed for CTCL, where there is an established population of malignant memory T lymphocytes that are tropic for the skin. This tropism is assumed to be determined by the place where lymphocytes first encountered antigen9 and to be mediated by an interplay of adhesion molecules and chemokines.7 However, the nature of the antigen(s) and the event that transforms the chronic polyclonal lymphoid infiltrate to a malignant monoclonal CTCL remains unknown.

We have also used reverse transcriptase PCR and we have detected IFNγ in 41% (95% CI, 18% to 67%) and TNFα in 59% (95% CI, 33% to 92%) of lesional biopsies of all stages of CTCL.9 These results are comparable to those reported for all CTCL patients by Asadullah et al for IFNγ (45%, 95% CI, 23% to 68%) and TNFα (95%, 95% CI, 75% to 100%).

Our model was introduced as a "framework for the systematic investigation of the roles played by IP-10 (and possibly other chemokines), selectins, integrins, and adhesion molecules in the biology of CTCL."11 We feel that the aggregate results of Asadullah et al support our proposal and are pleased that it is already functioning as a framework for expanding our knowledge of the CTCL biology. Like all models, it may require refinement and modification as additional information is accumulated.

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REFERENCES

Table 1. Frequency and Detection of IFNγ and TNFα in CTCL

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>IFNγ (%)</th>
<th>95% CI</th>
<th>TNFα (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patches</td>
<td>11</td>
<td>64</td>
<td>31-89%</td>
<td>91</td>
<td>59-100</td>
</tr>
<tr>
<td>Plaque</td>
<td>6</td>
<td>33</td>
<td>43-78%</td>
<td>100</td>
<td>54-100</td>
</tr>
<tr>
<td>Tumor</td>
<td>3</td>
<td>0</td>
<td>0-71%</td>
<td>99</td>
<td>29-100</td>
</tr>
<tr>
<td>All stages</td>
<td>20</td>
<td>45</td>
<td>23-68%</td>
<td>95</td>
<td>75-100</td>
</tr>
<tr>
<td>Healthy skin</td>
<td>8</td>
<td>0</td>
<td>0-37%</td>
<td>88</td>
<td>47-100</td>
</tr>
</tbody>
</table>
Interferon gamma and tumor necrosis factor alpha mRNA expression in mycosis fungoides progression [letter; comment]

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