Isoprinosine and Imuthiol, Two Potentially Active Compounds in Patients with AIDS-related Complex Symptoms

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Abstract

Isoprinosine and Imuthiol are immunomodulators with a unique effect on T-cells. The possibility of using them in treating patients with acquired immunodefi ciency syndrome related complex (ARC) was initially examined regarding their in vitro effects on peripheral blood mononuclear cells. In six ARC patients Isoprinosine (100 μg/ml) and Imuthiol (10 pg/ml) induced in vitro an early chromatin activation as measured by nuclear refringency test and potentiated phytohemagglutinin (5 μg/ml) in the same 20-min assay in the absence of fetal calf serum. In all patients an early phytohemagglutinin induced chromatin dispersion was observed with a dose related response before interleukin 2 production can occur. Isoprinosine and Imuthiol increased signifi cantly both the percentage and the absolute number of T4+ cells when peripheral blood mononuclear cells were incubated for 4 days in RPMI supplemented with 10% fetal calf serum. No changes in T8+ cells were noted.

Three homosexual ARC patients were then treated p.o. with Imuthiol (5–10 mg/kg/week) for 4 to 6 months. Without any deleterious effect a clinical improvement (in terms of adenopathy and opportunistic infection regression) and restoration of the response to recall antigens were observed in all three patients. One patient with less than 500 T4+ lymphocytes/mm3 exhibited a complete restoration of OKT profiles. In such patients clinical and immunological effects of Isoprinosine have already been reported by others. Altogether these preliminary results indicate that more data should be obtained on the effects of these two agents in ARC patients.

Introduction

There is presently no established therapy to restore immune function in patients with AIDS. In order to evaluate the use of immunomodulators in treating these patients, we have tested the in vitro effects of Isoprinosine, an inosinic compound (1), and Imuthiol (diethyldithiocarbamate), a sulfur containing organic compound (2). Isoprinosine has been reported to prevent viral infections (3) and, when adjusted to appropriate dosage, exhibits a synergistic effect in vitro on the proliferative response to mitogenic lectins (4). Isoprinosine and Imuthiol are both considered T-cell inducers and are responsible for an increase in OKT4+ cells (5–7).

In this study the in vitro effects of these agents in ARC patients are assessed in terms of early chromatin activation as measured by a nuclear refringency test (8) and of T-helper induction as measured by monoclonal antibodies of the OKT series (9). The influence of an in vivo treatment by Imuthiol on three ARC patients is presented here as a preliminary report.

Patients and Methods

Patients

Peripheral blood mononuclear cells from six homosexual men ages 25–35 years with ARC (diffuse adenopathy and opportunistic infections) have been studied in vitro. In these patients the mean value of T-cell subsets was 54.4% OKT3+, 20% OKT4+, and 35.8% OKT8+. The absolute number of OKT4+ cells was less than 500/mm3. The in vivo effects of Imuthiol were tested on three homosexual men ages 25–35 years, with ARC among which only one had a decrease in T4+ cells (102/mm3; T4/T8 ratio, 0.58). The others had more than 500/mm3 T4+ cells.

Lymphocytes from venous heparinized blood were purified by Ficoll-Hypaque (Pharmacia Laboratories) gradient centrifugation. Cells were washed in RPMI 1640 (Flow Laboratories) and spun at 150 × g to remove platelets. Final cell preparations were adjusted for all cultures to 106 cells/ml. They were more than 98% pure mononuclear cells (85% lymphocytes by morphological criteria).

Short term cultures were performed in RPMI 1640 without human serum or FCS. After 1 h preincubation at 37°C purified PHA (PHA HA 16; Wellcome Laboratories) was added at different concentrations from 1.25 to 10 μg/ml. Isoprinosine, 100 μg/ml (Laboratoire Delalande), and Imuthiol, 10 pg/ml (Institut Méreux), were added alone or in association with PHA. In all experiments the cells were incubated for 20 min only and then treated in order to observe the nuclear refringency modifications. The nuclear refringency technique has been described previously (8). Briefly the nuclear refringency index (NRI) is calculated on control and stimulated triplicates and the nuclear refringency test (NRT) is expressed as

\[
NRT = 100 \times \frac{NRI_{treated}}{NRI_{control}}
\]

A decreased nuclear refringency index reflects chromatin dispersion related to nuclear activation and the results are expressed as percentage of nuclear activation (8).

The blood mononuclear cells from the same patients were cultured during 4 days in RPMI added with 10% heat inactivated FCS (Grand Island Biological Company, Grand Island, NY), specolin G (100 IU/ml; Specia Laboratories), and streptomycin (100 μg/ml; Specia). The cells (1 × 106 viable cells/ml of supplemented RPMI 1640) were distributed at 300 μl/well in flat-bottomed microplates (Costar, Cambridge, MA). Equal volumes of inducer and medium were added in triplicate wells. After 4 days of incubation at 37°C in a humid atmosphere of 5% CO2, cell number and viability were determined (trypan blue exclusion test) by counts in a hemocytometer by phase-contrast microscopy on the pool of triplicate wells. Afterwards the cells were washed twice and resus-
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Modification of OKT4 lymphocytes in patients with NDS related complex symptoms after in vitro incubation with Isoprinosine or Imuthiol

OKT3 OKT4 OKT8

<table>
<thead>
<tr>
<th></th>
<th>OKT3</th>
<th>OKT4</th>
<th>OKT8</th>
<th>OKT4/OKT8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before culture</td>
<td>54.4 ± 4.6(^b)</td>
<td>20 ± 2.8</td>
<td>35.8 ± 3</td>
<td>0.57</td>
</tr>
<tr>
<td>Control</td>
<td>63.5 ± 6.5</td>
<td>24 ± 5.1</td>
<td>40 ± 5.2</td>
<td>331 ± 27</td>
</tr>
<tr>
<td>After 4 days of culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoprinosine (100 μg/ml)</td>
<td>72 ± 9.2</td>
<td>618 ± 80</td>
<td>38.7 ± 6.4</td>
<td>333 ± 55(^b)</td>
</tr>
<tr>
<td>Imuthiol (10 pg/ml)</td>
<td>76 ± 8.4</td>
<td>647 ± 72</td>
<td>41.5 ± 8.7</td>
<td>354 ± 58(^b)</td>
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</tbody>
</table>

\(^b\) Mean ± SD in six patients.

Results

Early PHA Response to PHA and Immunomodulators. The early response to PHA is not impaired in ARC patients and the dose related response to lectin is depicted in Chart 1.

As shown in Chart 2 Isoprinosine or Imuthiol alone is responsible for an early chromatin dispersion and a synergistic effect is observed between PHA and each of the immunomodulators.

T-Lymphocyte Subsets. In control samples a slight but not significant increase in OKT phenotypic expression is observed after 4-day culture. The T4/T8 ratio is not modified because all subsets are affected (Table 1).

After stimulation with Isoprinosine or Imuthiol a significant increase in T4* cells (67 and 78%, respectively, for Isoprinosine and Imuthiol) is responsible for an elevation of T4/T8 ratio. The T8* lymphocytes are not modified at the concentrations utilized in cultures.

Effects of Imuthiol on ARC Patients. Three patients with ARC have been tested. One of them, presenting a low T4* cell count (500/mm\(^3\)), has shown an increase in erythrocyte forming rosette-positive and T3* cells and an increase in the T4/T8 ratio (Table 2). This was concomitant with a restoration of the response to recall antigens and the disappearance of adenopathy.

In two other patients we also observed a clinical improvement (in terms of opportunistic infections and adenopathy regression), restoration of delayed cutaneous hypersensitivity, but no significant changes in the OKT profiles.

Discussion

Peripheral blood lymphocytes from ARC patients exhibit a sensitivity to PHA with a dose related effect. The PHA induced chromatin dispersion occurs as early as 20 min after stimulation, before IL2 production can be induced. This could explain that an early effect on chromatin can be observed without any proliferative response to PHA. Isoprinosine or Imuthiol are responsible for similar effects on chromatin which have been related previously to the induction of transcriptional state on the nucleus (8). The effects of these two immunomodulators on chromatin status can be responsible for the regulation of OKT4 phenotypic expression and the induction of IL2 by those compounds (10, 11). In normal subjects Imuthiol has been reported previously to transform null cells into OKT4* HLA-DR* cells (7). This mechanism could also be involved in ARC patients.

Regarding IL2 one hypothesis to explain the increased number

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Table 1
Modification of OKT4* lymphocytes in patients with AIDs related complex symptoms after in vitro incubation with Isoprinosine or Imuthiol

<table>
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\(^b\) Mean ± SD in six patients.

\(^b\) P < 0.01 by Wilcoxon test; ratios to control are 1.67 for Isoprinosine and 1.78 for Imuthiol.
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Table 2

Imuthiol treatment of a lymphadenopathy syndrome patient
A 26-year-old homosexual with LAS has been treated by Imuthiol, 5 mg/kg weekly during 16 weeks. Diffuse adenopathy and splenomegaly disappeared and a significant effect was observed on immunological parameters.

<table>
<thead>
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<th>Immunological parameters</th>
<th>Before</th>
<th>After</th>
</tr>
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<tr>
<td>OKT3</td>
<td>19</td>
<td>194</td>
</tr>
<tr>
<td>OKT4</td>
<td>10</td>
<td>102</td>
</tr>
<tr>
<td>OKT8</td>
<td>17</td>
<td>173</td>
</tr>
<tr>
<td>OKT4/OKT8</td>
<td>0.58</td>
<td>1.20</td>
</tr>
<tr>
<td>Erythrocyte forming rosette cells (%)</td>
<td>33</td>
<td>70</td>
</tr>
<tr>
<td>Delayed cutaneous hypersensitivity</td>
<td>0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

of IL2 receptors on lymphocytes from AIDS patients could be related to the defect in IL2 production, and the restoration of intrinsic production by those immunomodulators could modify the receptor expression of T-lymphocytes.

The effects of these two immunomodulating agents could be related to a regulation of the dysfunctioning between IL2 and its receptors and of the OKT4 phenotypic expression as well, these parameters belonging altogether to the characteristics of lymphocytes infection by LAV/HTLV-III.

From the therapeutic point of view a previous report has shown a beneficial effect of Isoprinosine in pre-AIDS patients (12). However, the best schedule of administration must still be established to escape immunodepression after long term treatment. Imuthiol has shown a short term improvement in three ARC patients without any side effects but it seems now too early to make conclusions.

Altogether these data indicate that more data should be obtained on these two immunomodulating agents regarding their effects in AIDS patients. Because of their absence of deleterious effects the use of these agents can be proposed in AIDS in association with direct antiviral agents and in the preventive treatment of seropositive subjects particularly when they present a decrease in T4+ subset. The drugs may also be beneficial to seropositive hemophiliacs.

Acknowledgments
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References
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