Immunological Effects of Retinoids

Péter GERGELY, Lilla CSÁKY and Phenzo GONZÁLEZ-CABELLO

Second Department of Medicine, Semmelweis University, Budapest and Buda Disttrict Children's Hospital, Hungary
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Vitamin A treatment (100,000 U daily) of systemic lupus erythematosus, non-Hodgkin’s lymphoma and chronic lymphocytic leukemia patients, children suffering from recurrent respiratory tract infections and healthy controls resulted in an enhancement of antibody-dependent cell-mediated cytotoxicity, natural killer activity and blastogenic response to mitogens.

In vitro, retinoids, depending on the concentration, stimulated mitogen- and interleukin-2-induced blastogenesis and lectin-dependent T cell cytotoxicity. Retinoids caused an early plasma membrane hyperpolarization of cells of various origin. This effect was similar to that seen by interferon alpha. Retinoids also slightly inhibited intracellular calcium accumulation.

(Key Words: immunostimulation, vitamin A, retinoids)

INTRODUCTION

Even though the protective role of vitamin A and other retinoids against infections and neoplasia has been demonstrated, the exact mechanism by which they exert this effect is not completely understood. Of numerous biological effects, the immunostimulatory activity of retinoids has been well established. Vitamin A and its derivatives enhance both humoral and cell-mediated immune reactions, increase lymphokine secretion, and possess immune regulatory activity.

Recently, we have reported that vitamin A treatment increase mitogen-induced blast transformation, NK and K cell activity in a number of disorders that display an impairment of cell-mediated immunity.

MATERIALS AND METHODS

Patients

Patients suffering from chronic lymphocytic leukemia (CLL), non-Hodgkin’s lymphoma (MHL), systemic lupus erythematosus (SLE), children suffering from recurrent upper respiratory tract infections and healthy controls were studied. They were taking 100,000 U vitamin A (EGIS, Hungary) capsules daily for 2 weeks. Blood was drawn before and after 2 weeks of vitamin A therapy.

Cells

Human peripheral blood lymphocytes (PBL) were separated from heparinized venous blood. Mouse thymocytes and spleen cells were separated from 3–4 week old Balb/c female mice. Daudi and U 937 cells were maintained in culture.

Cell-mediated immune reactivity

Antibody-dependent cell-mediated cytotoxicity (ADCC) was assessed by the method of Perlmann and Perlmann (11) with slight modifications. In brief, 51chromium-labelled, antibody-coated chicken red cells were used as targets; effector to target cell ratio was 2.5:1 and 5:1, incubation time 4 hours. The reaction was terminated by centrifugation, and radioactivity of the supernatant was measured in a gamma counter. Target cell damage was expressed as cytotoxicity index (CI%).

Natural killer (NK) cell activity was measured using 51chromium-labelled K-562 targets at 50:1 and 25:1 effector to target cell ratio in a
4 hour assay. Activity was expressed as cytotoxicity index (CI%).

Lectin-dependent cell-mediated cytotoxicity (LDCC) was measured by detachment from the monolayer of \(^{3}H\).thymidine prelabelled HEp-2 target cells in flat bottomed microplates (Greiner, FRG). LDCC activity was evaluated in a 24h assay at 50:1 effector to target cell ratio in the presence of 25ug/ml concanavalin A (Con A, Pharmacia, Sweden). LDCC was expressed as the percentage increase of cytotoxicity attributable to the presence of Con A. Natural cell-mediated cytotoxicity (NMC) was calculated taking the medium control as baseline.

Lymphocyte transformation: PBL were cultured in Greiner (FRG) microplates (2 × 10° cells/well) in complete RPMI 1640 medium. Two or 10ug/ml phytohaemagglutinin (PHA, Leucoagglutinin, Pharmacia, Sweden) or 10 or 25ug/ml Con A (P farmacia, Sweden) were used as mitogens. Human recombinant interleukin 2 (IL-2, Behring, FRG) was used at a concentration of 10U/ml. The cultures were kept at 37°C for 72 hours. Eight hours before harvesting, 0.5 uCi \(^{3}H\).thymidine (Amersham, UK) was added to each well. After termination, the cultures were harvested by an automated sample harvester (Skatron, Norway). Isotope determinations were made in a Nuclear Isocap 500 counter.

Membrane potential was measured by the uptake of the fluorescent dye, bis-oxonol (Molecular Probes, USA) as described earlier (6). Two minutes after the addition of the dye, the fluorescence intensity of 10⁴ cells was checked in a FACScan (Becton-Dickinson, USA) flow cytometer.

Intracellular calcium was measured by uptake of the fluorescent indicator dye, Fluo-3 (Molecular Probes, USA) after stimulation of PBL by Con A. The change in fluorescence intensity was assessed in the cytometer.

Retinoids: retinoic acid (RA), retinol (ROH), and retinal (RAL) were purchased from Sigma, USA. Interferon alpha (IFN) was from Behring, FRG.

Statistical analysis was made by the Student's t test.

RESULTS

Effect of vitamin A treatment on the cellular immune reactivity

Vitamin A treatment resulted in an increase in the mitogen response to PHA and Con A (Table 1). ADCC and NK activity significantly increased at both effector to target cell ratios after treatment (Table 2).

In vitro effect of retinoids on the Con A and IL-2 induced blastogenesis

Both retinoic acid and retinal inhibited the response induced by Con A or IL-2 at higher concentrations. At lower concentrations this inhibitory effect disappeared. At even lower concentrations they exerted a significant stimulatory effect (Table 3).

In vitro effect of retinol on the LDCC activity

Retinol enhanced LDCC activity of human PBL at concentration of 10-7 M (Table 4), whereas decreased "spontaneous" cytotoxic (NMC) activity.

Effect of retinoids and IFN on the membrane potential

IFN hyperpolarized all cells. This effect was more pronounced using higher concentration (Table 5). Retinoic acid, retinol and retinal also dose-dependently hyperpolarized the cell membrane. At the same molar concentration retinal seemed to be more potent than retinoic acid; the effect of retinol was the slightest. When retinoids and IFN was given simultaneously, the hyperpolarization became more prominent. No antagonizing effect was observed.

Effect of retinoids on the intracellular Ca content

Neither retinal nor retinoic acid (at concentration of 10-6 M) induced anu measurable intracellular calcium accumulation during 20 min after addition to human PBL. They rather inhibited Con A-induced increase in intracellular Ca uptake. The effect was not seen at lower concentrations (data not shown).

DISCUSSION

Treatment with vitamin A for two weeks (100,000 U daily) resulted in an increase of cytotoxic (ADCC, NK activity and mitogenic response to PHA and Con A. These in vivo immunostimulatory effects are in accordance with those obtained earlier (5, 19). The in vitro effects, however, are more contradictory. Both retinoic acid and retinal suppressed Con A- and IL-2-induced mitogenic response of PBL in
Table 1 Effect of vitamin A treatment on the mitogen response of lymphocytes (25μg/ml Con A; mean c.p.m. ± SEM)

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Before therapy</th>
<th>After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>1324 ± 350</td>
<td>2168 ± 857*</td>
</tr>
<tr>
<td>CLL</td>
<td>3931 ± 816</td>
<td>10190 ± 2278*</td>
</tr>
<tr>
<td>NHL</td>
<td>1617 ± 291</td>
<td>2736 ± 961*</td>
</tr>
<tr>
<td>SLE</td>
<td>1700 ± 602</td>
<td>5293 ± 2990**</td>
</tr>
<tr>
<td>Recurrent infection</td>
<td>1570 ± 320</td>
<td>3242 ± 458*</td>
</tr>
</tbody>
</table>

* significant (p<0.05)
** significant (p<0.01)

Table 2 Effect of vitamin A treatment on the cytotoxic activity of K and NK cells (mean C.I. ± SEM)

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Before therapy</th>
<th>After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>18.11 ± 1.7</td>
<td>44.31 ± 6.5*</td>
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<tr>
<td>NK</td>
<td>29.92 ± 1.4</td>
<td>34.38 ± 2.4</td>
</tr>
<tr>
<td>CLL</td>
<td>10.01 ± 2.7</td>
<td>19.10 ± 4.8*</td>
</tr>
<tr>
<td>NK</td>
<td>18.76 ± 1.5</td>
<td>28.65 ± 2.4*</td>
</tr>
<tr>
<td>NHL</td>
<td>26.50 ± 1.9</td>
<td>31.80 ± 1.9</td>
</tr>
<tr>
<td>NK</td>
<td>18.34 ± 0.9</td>
<td>32.78 ± 2.5*</td>
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<tr>
<td>SLE</td>
<td>15.91 ± 1.5</td>
<td>41.90 ± 2.3*</td>
</tr>
<tr>
<td>NK</td>
<td>13.68 ± 1.1</td>
<td>23.35 ± 1.7*</td>
</tr>
<tr>
<td>Recurrent infection</td>
<td>17.70 ± 2.0</td>
<td>27.70 ± 3.1*</td>
</tr>
<tr>
<td>NK</td>
<td>24.50 ± 2.1</td>
<td>33.21 ± 4.0*</td>
</tr>
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</table>

* significant (p<0.05)

Table 3 In vitro effect of retinoids on the Con A·(10μg/ml) and IL-2·(10U/ml) induced blastogenesis of human PBL (mean c.p.m., n:5)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>none</th>
<th>IL-2</th>
<th>Con A</th>
<th>Con A + IL-2</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>230</td>
<td>1340</td>
<td>10520</td>
<td>18950</td>
</tr>
<tr>
<td>RA (10⁻⁶)</td>
<td>198</td>
<td>840</td>
<td>4455</td>
<td>6679</td>
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<tr>
<td></td>
<td>167</td>
<td>958</td>
<td>6247</td>
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<td></td>
<td>189</td>
<td>2077</td>
<td>13245</td>
<td>22534</td>
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<tr>
<td>RAL (10⁻⁶)</td>
<td>86</td>
<td>122</td>
<td>340</td>
<td>778</td>
</tr>
<tr>
<td></td>
<td>123</td>
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<td></td>
<td>197</td>
<td>1976</td>
<td>15616</td>
<td>20043</td>
</tr>
</tbody>
</table>
concentrations near to plasma level. This may be explained by their effect upon protein kinase C, a key enzyme in lymphocyte blastogenesis (8, 12). At much lower concentrations, however, both derivatives enhanced thymogenic effect. This is in accordance with that of other (4, 18). The increase in LDCC activity may be a direct effect upon T cells.

The alteration of plasma membrane potential is one of the earliest consequences of lymphocyte activation. All retinoids hyperpolarized the plasma membrane of lymphocytes of various origin and cells of myelomonocyte line U937. This effect was dose-dependent, resembling to that induced by interferon alpha. This effect of retinoids, albeit not unexpected (1, 9, 10), has not been shown earlier. In many test sytems, IFN and retinoids exerts a reciprocal action (7, 14, 15). In our hands, they activated all kind of cells, and no antagonizing effects were seen. Despite the early activation signals produced by retinoids, there was no increase in intracellular calcium content. In fact, retinoids slightly inhibited calcium accumulation induced by mitogens. This effect of retinoids has been demonstrated in other test systems (2, 16).

Therefore, the above shown in vitro effects of retinoids may not provide a sufficient explanation for the dramatic immune stimulatory activity observed in vivo.

**REFERENCES**


6) Grimley PM, Asalar A: Early plasma membrane