Suppression of mitogen- and antigen-induced lymphocyte proliferation by lanthanides

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Summary. Lanthanide ions (Ln^{3+}) inhibited the proliferative response of human lymphocytes to various polyclonal mitogens and the 'purified protein derivative' (PPD) of the tuberculin antigen. Of the four Ln^{3+} ions tested lanthanum (La^{3+}) was the strongest inhibitor; erbium (Er^{3+}) and lutetium (Lu^{3+}) were only weakly active, while samarium (Sm^{3+}) had intermediate potency. At a concentration of 1 mM, La^{3+} almost completely inhibited the uptake of [^{3}H]-thymidine by lymphocytes exposed to mitogenic agents. Trypan blue exclusion tests confirmed that the La^{3+} ions were not toxic. These findings may bear upon the reported anti-inflammatory properties of the lanthanides.

Key words. Lanthanide; lymphocyte; cell division; inflammation.

Ions of the lanthanide series of elements (at. no. s 57–71) have remarkable biochemical, biological and pharmacological properties [1–6]. Many, but not all, of these properties follow from the ability of Ln^{3+} ions to replace Ca^{2+} isomorphously in biological systems.

The literature contains persistent reports attributing anti-inflammatory activity to the lanthanides. Among the first of these was that of Jancso [7] who noted that various Ln^{3+} ions, and certain of their complexes, inhibited angiotaxis and oedema in rats following the injection of inflammatory substances. Similar properties were subsequently reported by others [4, 8–11].

The observations presently lack an explanation, although it is possible to implicate the supressive effects that Ln^{3+} have upon the reticuloendothelial system [12, 13] and upon certain functions of cultured polymorphonuclear leucocytes [14–16]. In the present work, we have explored the possibility that Ln^{3+} interfere with lymphocyte activation. This seemed a promising area for investigation as lymphocyte activation is well known to require the influx of Ca^{2+} ions from the extracellular environment [17–21], while Ln^{3+} ions are potent Ca^{2+}-blockers [2, 6, 22–25].

Materials and methods

Human donors were identified as tuberculin-positive by a delayed skin reaction following intradermal challenge. Mononuclear cells were isolated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation of their heparinized, venous blood. After washing, cells were resuspended in RPMI-1640, supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), 2-mercaptoethanol (50 µM), HEPES (25 mM) and 10% foetal bovine serum at a concentration of 10^6 cells/ml. Aliquots (100 µl) of this suspension were dispensed into wells on a 96-well plate.

Lanthanide chlorides were dissolved in 10^{-4} M HCl to form stock solutions of 10 mM concentration. These were then diluted in culture medium and added to the cell cultures at the indicated concentrations. This procedure did not alter the pH of the culture medium. At this time one of the mitogens concanavalin A (Con A), pokeweed mitogen (PWM) and phytohaemagglutinin (PHA), or the PPD antigen were added to certain wells.

Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2, 95% air, for 3 days, for mitogen-stimulated cultures, and for 5 days for antigen-stimulated cultures. Four hours prior to harvesting the cells, 0.5 µCi of [^{3}H]-thymidine (Radium Chemie, Zurich, Switzerland; sp. act. 26 Ci/mmol) was added to each culture. Cells were harvested on glass fibre discs and the incorporation of [^{3}H]-thymidine determined by liquid scintillation. Results are expressed as the means ± standard error of the cpm of radioactivity incorporated by the cells.

Results and discussion

Ln^{3+} ions inhibited the proliferation of human peripheral blood lymphocytes responding to either polyclonal mitogens (Con A, PWM, PHA) or antigen (PPD). La^{3+} ions inhibited responses to all four agents with approximately equal efficiency, whereas suppression by Sm^{3+} ions was strongest for PPD and least effective with PHA (figs 1 and 2). In each case La^{3+} proved a stronger inhibitor than Sm^{3+}, while Er^{3+} and Lu^{3+} ions were only weakly active (figs 1–4). The relative inactivity of the latter two Ln^{3+} ions serves as an internal control demonstrating that inhibition by Sm^{3+} and La^{3+} has specificity.

Inhibition of proliferation by La^{3+} and Sm^{3+} occurred within the unusually narrow concentration range of 10^{-4} M–10^{-3} M (figs 1–3). However, inclusion of 10^{-3} M La^{3+} ions in the culture medium almost completely suppressed thymidine incorporation by lymphocytes, regardless of the mitogenic stimulus. Similar concentrations of La^{3+} are necessary for inhibition of Ca^{2+}-dependent cellular responses in other types of non-excitable cell [26–28]. Furthermore, this concentration of

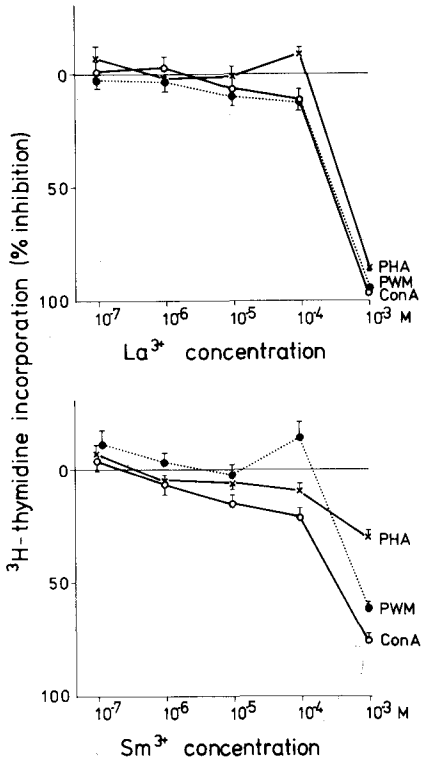

Figure 1. Effects of La^{3+} and Sm^{3+} upon the mitogen responsiveness of lymphocytes (day 3).

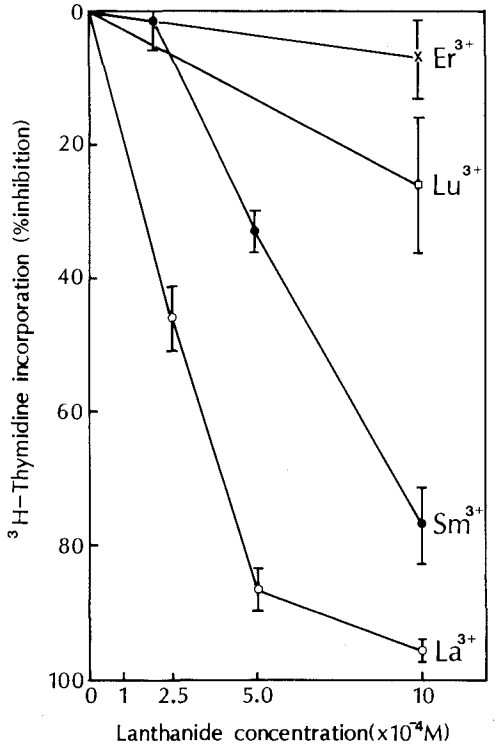

Figure 3. Lanthanide inhibition of the lymphocyte proliferative response to the PPD antigen.

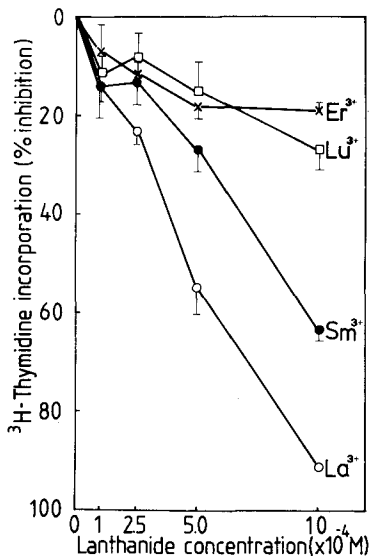

Figure 2. Lanthanide inhibition of the lymphocyte proliferative response to Con A.

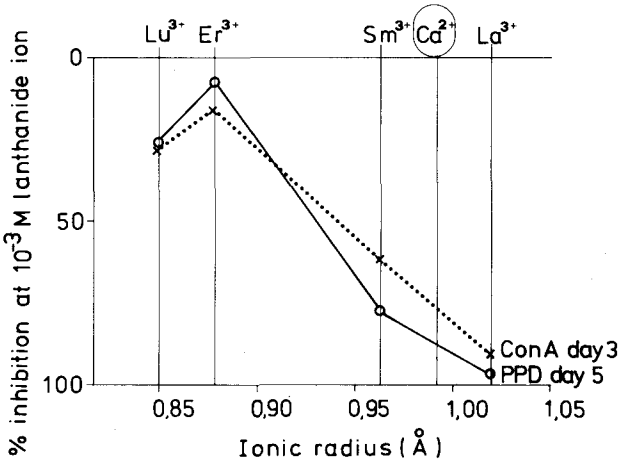

Figure 4. Relationship between ionic radius and inhibition of lymphocyte proliferation. Ionic radii are taken from Shannon[33], assuming a coordination number of 6.

La^{3+} has been previously found to inhibit 'spotting' and 'capping' of immunoglobulins on lymphocyte surfaces[29].

Trypan blue exclusion tests confirmed that lymphocytes retained 85% viability both in the presence or absence of Ln^{3+} ions, thus eliminating toxicity as a trivial explanation of the phenomenon. In figure 4, the inhibitory potency of each Ln^{3+} ion is compared to its radius and to that of Ca^{2+}. This plot clearly identifies the largest Ln^{3+} ions, whose radii are closest to that of Ca^{2+}, as the strongest inhibitors. While consistent with a mode of inhibition that involves antagonizing Ca^{2+} influx, direct

measurements will be necessary to confirm this. If Ln^{3+} ions competitively block Ca^{2+} ion uptake in this system, the presence of millimolar concentrations of Ca^{2+} in culture medium would explain the ineffectiveness of low concentrations of Ln^{3+} ions.

Inhibition of lymphocyte activation in vivo could contribute to the purported anti-inflammatory properties of the lanthanides. This suggestion is supported by the observation that several steroidal and non-steroidal anti-inflammatory drugs inhibit lymphocyte proliferation in vivo [30, 31]. Gold chloride shares this property [32], an interesting finding in light of the antiarthritic properties of gold drugs. More than one author has speculated that lanthanides might find therapeutic use in arthritis [5, 6, 10, 11].

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Absence of immunosuppression in DBA/2 mice vaccinated with *Trypanosoma cruzi* treated with actinomycin-D

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Summary. The subcutaneous (s.c.) vaccination of DBA/2 mice with 4 weekly doses of 3×10^7 living metacyclic forms of *T. cruzi*, Y strain, obtained from culture in axenic medium and treated for 24 h with actinomycin-D (50 μg/10^7 parasites), a drug that promotes an irreversible blockade of the parasite replication, do not induce any detectable degree of humoral and cellular immunosuppression as assessed by a) the production of anti-SRBC antibodies, b) the permanence of delayed cutaneous reaction to *T. cruzi* antigen, to PPD and DNCB and c) the degree of blastogenic transformation of spleen lymphocytes in the presence of the specific antigen.

Key words. T. cruzi; immunology; *T. cruzi* vaccination; DBA/2; actinomycin-D; immunosuppression.

Acute infection of mice with *T. cruzi* usually induces a state of immunosuppression, varying in duration and depending on the biological characteristics of the parasite, the dose and route of its inoculation and the genetic constitution of the host [2, 4, 8–11]. Several hypotheses have been formulated to try to explain the mechanism of this suppression [1, 5–7].

A potent immunogen is obtained by treatment of viable metacyclic culture or blood forms of *T. cruzi*, Y strain, with an adequate dose of actinomycin-D, a drug that