Selective Modulation of Antibody Response and Natural Killer Cell Activity by Purine Nucleoside Analogues

Teresa Priebe, Osama Kandil, Melita Nakic, Bih Fang Pan, and J. Arly Nelson

Department of Experimental Pediatrics, University of Texas-M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

ABSTRACT

Analogue that are poor substrates for adenosine deaminase or purine nucleoside phosphorylase may mimic immunodeficiencies associated with the enzyme deficiencies, and their activities may be directed toward selected lymphocyte subpopulations. Four analogues were studied for their effects on primary antibody response to either a T-dependent (sheep erythrocytes) or T-independent (trinitrophenyl-conjugated Escherichia coli lipopolysaccharide) antigen as well as effects on T-cytotoxic and natural killer cell activities in mice. The nucleosides were: an adenosine analogue, tubercidin; two deoxyadenosine analogues, 2-chloro, 2'-deoxyadenosine and 2-fluorodeoxyadenosine-5'-phosphate; and a deoxyguanosine analogue, 9-ß-D-arabinosylguanine. Drugs were given i.p. once daily for 3 consecutive days. Immune responses were determined in spleen cell suspensions 1 day after the last dose. Tubercidin inhibited both T-cytotoxic and natural killer cell activities at doses that did not reduce primary antibody response, whereas the reverse was true for 2-chloro, 2'-deoxyadenosine and 2-fluorodeoxyadenosine-5'-phosphate. At higher doses, T-cytotoxic lymphocytes appeared to be more sensitive than natural killer cells to the deoxyadenosine analogues. 9-ß-D-Arabinosylguanine did not selectively inhibit the immune responses at doses that clearly reduced the yield of spleen lymphocytes. Assuming the analogues mimic endogenous nucleosides, the results suggest that natural killer cells are more sensitive to adenine than are those cells responsible for primary antibody response, whereas the reverse is true for deoxyadenosine.

INTRODUCTION

The biochemical bases for the immunodeficiencies associated with inherited deficiency of ADA2 or PNP are unknown (1). Several plausible hypotheses relate to accumulation of the substrates for ADA (Ado and dAdo) or PNP (dGuo). Testing these hypotheses by administering these nucleosides to normal animals is hampered by their rapid metabolism by ADA or PNP. The potent ADA inhibitor DCF has been used alone and in combination with ADA substrates in attempts to mimic the severe combined immune deficiency syndrome in animals (2, 3). A corresponding potent and selective inhibitor of PNP has not been developed; however, 8-aminoguanosine (4), 8-aminoguanosine (5), and the compound synthesized by Klein (6) have been used for this purpose. An alternative approach is to use nucleoside analogues that are poor substrates for ADA or PNP. Ideally, such analogues would be metabolized by other enzymes that utilize endogenous ADA or PNP substrates, and they would share mechanisms of action with the endogenous nucleosides. Whether such ideal conditions can be met with available nucleoside analogues is not known. To test whether they can be met, we have evaluated selected analogues in vivo to determine whether features of ADA or PNP deficiency (or inhibition) can be simulated. We evaluated an Ado analogue (Tub), two dAdo analogues (ClAdo, FaraAMP), and a dGuo analogue (araGu) in mice as potential modulators of immune response. Effects of these agents on primary antibody response to a T-cell-dependent (SRBC) and a T-cell independent (TNP-LPS) antigen as well as NK and CTL are reported herein. The results indicate that selective modulation of humoral and cellular immune response can be achieved with nucleoside analogues. Preliminary reports of these data have been presented (7, 8).

MATERIALS AND METHODS

Animal Treatments. Primary antibody response to SRBC or to TNP-LPS was evaluated in male AKR/J mice. Animals were treated i.p. with 108 sheep erythrocytes or with 10 µg of TNP-LPS (Sigma Chemical Co., St. Louis, MO) in 0.3 ml of normal saline. The mice then received various i.p. doses of purine nucleoside analogues: Tub (Sigma); araGu (Calbiochem-Behring Corp., La Jolla, CA); ClAdo; and FaraAMP (Drug Synthesis and Development Branch of the National Cancer Institute) on Days 1, 2, and 3 following antigen administration. On Day 4, the spleens were removed from groups of three mice each and evaluated for antibody formation as described below. CTL were generated in male C3H/He mice with i.p. administration of 2 x 106 P815 mastocytoma cells. The purine nucleosides were then administered i.p. once daily on Days 9, 10, and 11. The spleens were harvested on Day 12 and evaluated for CTL. Similarly, to assess NK activity, male C3H/He mice were treated with nucleoside analogues once daily for 3 days, and on Day 4 spleens were removed for NK assay.

Antibody Plaque Assay. Animals were sacrificed by CO2 asphyxia, and spleen cell suspensions were prepared in RPMI-1640 medium by passage through stainless steel mesh sieves. The spleen cells (0.5 x 106 cells) in 100 µl were then added to 50 µl of 10% SRBC in 0.5% gelatin. To evaluate antibody formation in animals immunized with TNP-LPS, the SRBC were first conjugated with TNP (9). Following addition of 50 µl of guinea pig complement which had been adsorbed with SRBC, the contents of each sample were transferred to fill both chambers of a Cunningham slide. The long edges of each chamber were sealed with paraffin wax, and the slides were incubated for 1 h at 37°C. Areas of lysis were then evaluated under x10 magnification (10).

Cytotoxicity Assays. The 51Cr release assay was based on the technique described by Brunner et al. (11). A Moloney virus-induced lymphoma of an A/Sn origin cell line, YAC-1, was used as a target for NK activity, and P815 mastocytoma of DBA/2 origin was used for the CTL assay.

Tumor Cells. Tumor cells were maintained as stationary suspension cultures in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 1% penicillin and streptomycin. Target cells (5 x 104) were incubated for 1 h with 0.2 mCi of 3H Cr (sodium chromate; American, Arlington Heights, IL), washed 3 to 4 times with RPMI 1640, and resuspended in the above medium to a concentration of 106 cells/ml. Effector spleen cell suspensions were prepared in RPMI 1640 as described above. Erythrocytes and granulocytes were removed by ficoll-Hypaque (Pharmacia, Inc., Piscataway, NJ) density gradient centrifugation. Aliquots of 0.1 ml of labeled target cells were mixed with 0.1

Received 12/23/87; revised 4/27/88; accepted 6/6/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. 1Supported by Grant HD-13951 from the Institute for Child Health and Development and Grant CA-28034 from the National Cancer Institute, the NIH.

2To whom requests for reprints should be addressed at Department of Experimental Pediatrics, University of Texas-M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

3The abbreviations used are: ADA, adenosine deaminase (EC 3.5.4.4); Ado, adenosine; araGu, 9-ß-D-arabinosylguanine; dAdo, deoxyadenosine; ClAdo, 2-chloro, 2'-deoxyadenosine; CTL, cytotoxic lymphocytes; DCF, 2'-deoxycoformycin; FaraAMP, 2-fluorodeoxyadenosine-5'-phosphate; PNP, purine nucleoside phosphorylase (EC 2.4.2.1); Tub, tubercidin; NK, natural killer cells; LU, lytic unit; SRBC, sheep red blood cells; TNP-LPS, trinitrophenyl-conjugated Escherichia coli lipopolysaccharide; dGuo, deoxyguanosine; FITC, fluorescein isothiocyanate; LD50, dose lethal to 10% of the population.

Downloaded from cancerceres.sacnjournals.org on July 17, 2017. © 1988 American Association for Cancer Research.
ml of spleen cells in effector: target ratios of 100:1, 50:1, 25:1, and 12.5:1 in U-shaped 96-well microtiter plates and incubated for 4 h at 37°C in a 5% CO2 incubator. After incubation, the microplates were centrifuged, and the supernatant was evaluated for 51Cr release in a gamma counter.

Experimental results were expressed as lytic units (LU), defined as the number of effector cells, extrapolated from the dose-response curve, that was required to achieve n% of specific target cell lysis (12).

Immunofluorescence Microscopy. The distributions of various subpopulations of lymphocytes were evaluated in the spleens of the mice as follows. The lymphocytes were washed and resuspended in phosphate-buffered saline at a cell concentration of 107/ml. Aliquots of 100 μl were then incubated for 30 min at 4°C with 50 μl of 2% FITC-conjugated antibodies specific for Lyt-2 monoclonal antibody (Beckton Dickinson & Co., Mountain View, CA) or with FITC-conjugated anti-mouse immunoglobulin. Presence of L3T4 antigen was determined by indirect immunofluorescence as follows. Aliquots (100 μl) of the lymphocyte suspensions were incubated with hybridoma supernatants containing rat monoclonal antibody specific for L3T4 determinants (13). After washing, the cells were incubated with FITC-coupled goat anti-rat immunoglobulin antibodies (adsorbed with mouse proteins to reduce cross-reactivity; Cappel Laboratories, Westchester, PA) at 4°C for an additional 30 min. The cells were then washed 3 times, resuspended in 40 μl of saline solution, and analyzed by immunofluorescence microscopy.

RESULTS

A reciprocal relationship exists between effects of the purine nucleoside analogues studied on primary antibody response and NK cell activity (Fig. 1). Specifically, Tub inhibited NK cell activity but produced an apparent stimulation of primary antibody response, whereas the reverse was true for the deoxynucleosine analogues, CldAdo and FaraAMP. Effects of these agents on primary antibody response to either a T-dependent (SRBC) or T-independent (TNP-LPS) antigen were qualitatively and quantitatively similar. The deoxynucleosine analogue, araGua, did not significantly alter these immune responses. The higher doses of each of the purine nucleoside analogues approximated the whole-animal LD50. Accordingly a clear dose-response relationship was observed over the dose range used when the numbers of spleen lymphocytes obtained at the time of animal sacrifice were plotted as a function of dose (Fig. 2). Thus, failure of araGua to alter the immune parameters measured was not due to inadequate drug delivery, since the higher dose (300 mg/kg) clearly reduced the yield of spleen lymphocytes. Control C57BL/6 mice have a higher number of spleen lymphocytes than AKR/J and C3H/He mice (results for which were combined in Fig. 2); however, the dose-response relationships for these purine nucleosides were similar for all three mouse strains used.

Tub reduced both CTL and NK cell activity (Fig. 1; Table 1). Effects of CldAdo and FaraAMP on the cytotoxic cell responses were qualitatively similar; i.e., lower doses appeared to enhance CTL activity slightly, whereas higher doses produced inhibition of this response (Table 1). In contrast, NK cell activity was enhanced even at the highest doses used. AraGua did not significantly alter the NK or CTL responses in these experiments. The effects of the adenosine analogue, Tub, on primary antibody response in these experiments (i.e., Fig. 1) are qualitatively similar to that for the potent ADA inhibitor, DCF (data not shown; Ratech et al., Ref. 3). Specifically, these compounds produce apparent enhancement of primary antibody response when administered as described in the legend to Fig. 1, i.e., beginning 1 day after antigen administration. If Tub treatment is initiated prior to the time of antigen administration, marked inhibition of primary antibody response is observed (Fig. 3) as

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg/day)</th>
<th>LUs10^6 cells</th>
<th>LUs/spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>42.9 ± 2.6*</td>
<td>777 ± 130</td>
</tr>
<tr>
<td>Tub</td>
<td>2</td>
<td>44.8 ± 5.0</td>
<td>558 ± 64</td>
</tr>
<tr>
<td>4</td>
<td>21.1 ± 1.6</td>
<td>189 ± 15</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>13.4 ± 3.5</td>
<td>76 ± 10</td>
<td></td>
</tr>
<tr>
<td>CldAdo</td>
<td>4</td>
<td>60.8 ± 8.1</td>
<td>758 ± 86</td>
</tr>
<tr>
<td>12.5</td>
<td>54.1 ± 1.5</td>
<td>645 ± 122</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>31.8 ± 5.2</td>
<td>310 ± 108</td>
<td></td>
</tr>
<tr>
<td>FaraAMP</td>
<td>75</td>
<td>49.0 ± 9.8</td>
<td>470 ± 82</td>
</tr>
<tr>
<td>150</td>
<td>73.9 ± 3.0</td>
<td>508 ± 4</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>33.4 ± 2.1</td>
<td>79 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>araGua</td>
<td>150</td>
<td>45.9 ± 13.8</td>
<td>659 ± 53</td>
</tr>
<tr>
<td>300</td>
<td>48.8 ± 1.2</td>
<td>323 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Effects of purine nucleoside analogues on CTL activity

Male C57BL/6 mice were treated with 2 x 10^7 P815 mastocytoma cells. Drugs were administered i.p. once daily 9, 10, and 11 days later. On the 12th day, spleen lymphocytes were evaluated for CTL activity as described in "Materials and Methods."
PURINE NUCLEOSIDE ANALOGUES

Fig. 3. Time-effect relationship for Tub effects on primary antibody response in AKR/J mice. Tub was administered once daily (4 mg/kg/day) for 3 consecutive days beginning on the day indicated. Mice were immunized with SRBC on Day 0, and primary antibody response was evaluated by plaque assay on Day 4 as described in "Materials and Methods." □, plaques/10^6 cells; ▼, plaques/spleen. The experiment shown is representative of two separate experiments. The simultaneous mean control values ± SE were: B-cells, 44 ± 4; non-B-cells, 45 ± 4; Lyt-2, 8.3 ± 1.2; L,T4, 23 ± 3.

Fig. 4. Effects of Tub on spleen lymphocyte subpopulations in AKR/J mice. Groups of three mice each were given SRBC on Day 0, and treated mice then received Tub (4 mg/kg/day) on Days 1, 2, and 3. Spleens were harvested for lymphocytes and the numbers of cells expressing surface immunoglobulin, Lyt-2, and L,T4 were determined by immunofluorescence microscopy. The results shown are from an experiment in which the pooled spleens of the three mice per group were used and compared with the simultaneous control group. The mean control values ± SE (numbers of lymphocytes x 10^6 per spleen, n = 6) were: B-cells, 44 ± 4; non-B-cells, 45 ± 4; Lyt-2, 8.3 ± 1.2; L,T4, 23 ± 3.

was reported for DCF by Ratche et al. (3). T-cells appear to be reduced in number at an earlier time after beginning the drug treatment than are B-cells; however, the time-course for Tub effects on the T-lymphocyte subpopulations (i.e., L,T4 and Lyt-2) were similar (Fig. 4).

DISCUSSION

Purine nucleosides appear to be highly cytotoxic, especially to lymphocytes (14–17), and are thought to play a role in ADA and PNP deficiencies associated with immune dysfunctions (18, 19). For this reason, investigators have explored mechanisms of nucleoside toxicity and effects on a variety of normal and malignant lymphoid cells. Several mechanisms for nucleoside cytotoxicity have been proposed. For example, deoxynucleosides alter intracellular deoxynucleoside triphosphate concentrations (20–22), inhibit S-adenosylhomocysteine hydrolase activity (23, 24), and produce single-strand breaks in DNA (25, 26). In some cases, selective toxicity of deoxynucleosides and their analogues toward malignant T- and B-lymphoblasts (27–32) has been shown to relate to a higher accumulation and lower rate of nucleotide catabolism in T- versus B-cell lines (33–34). Ado and its analogues also stimulate cyclic AMP production via an Ado receptor which has been described in cell membranes of erythrocyte-rosetting and null cells (35). In the immune system, cyclic AMP appears to act as a negative signal regarding cell proliferation and mature cell function (36).

The majority of humoral responses are thymus dependent; however, primary response to polysaccharides or proteins with identical subunits can occur in the apparent absence of T-cells (37). To determine if purine nucleoside analogues alter humoral in vivo immune response mediated via T-cells, antibody production against SRBC and TNP-LPS antigens has been evaluated. The purine nucleoside analogues exhibited no marked selectivity toward a T-dependent or T-independent antigen (Fig. 1). This implies that the drug effects may occur via B-cells directly or other accessory cells. Ratech et al. (3) and Nicholson et al. (38) reported a similar phenomenon for DCF and suggested the involvement of suppressor cells in regulation of humoral response by DCF. In view of this, enhancement of antibody formation by Tub might be a result of the drug’s inhibiting suppressor cells.

Several in vitro and in vivo studies have indicated NK cells to regulate humoral response induced by polyclonal mitogens or specific antigens (39, 40). Robles et al. (41) demonstrated in vitro the capacity of binding and lysing of LPS-activated B-cell blasts by unstimulated murine NK cells. In vivo, murine-stimulated NK cells had an inhibitory effect on primary antibody response against SRBC and the T-independent antigens, TNP-Ficoll and TNP Brucella abortus (42, 43). The observed reciprocal relationship between NK cell activity and primary antibody response (Fig. 1) is in agreement with these reports. Lytic activity of NK cells (44–46) and T-cytotoxic lymphocytes (47, 48) is inhibited by a number of agents that increase intracellular concentrations of cyclic cAMP, i.e., Ado and Ado analogues (49–51). It is tempting, therefore, to speculate that, under the conditions of drug treatment used herein, the Ado analogue (Tub) simply behaves as an agonist for a stimulatory adenosine receptor (i.e., A1 receptor) for NK cell activity, whereas CldAdo and FaraAMP mediate their effects via another adenosine receptor (i.e., A3 receptor) for NK cell activity, whereas CldAdo and FaraAMP mediate their effects via another adenosine receptor (i.e., A2 receptor) for NK cell activity. While investigating the Ado analogues 3-deazadenosine and 5’-methylthioadenosine, Fredholm et al. (52) reported that inhibition of methylation may also be critical for the effects of such agents on NK cell activity. CldAdo and FaraAMP enhanced NK cell activity and suppressed the humoral response (Fig. 1). These effects may be attributed to inhibition of suppressor cells but not helper cells, since similar effects have been noted with dAdo (53). The deoxynucleosine analogue araGua did not affect murine NK cell lytic function, which was also reported for deoxyguanosine by Varey et al. (54).

Effects of the purine nucleoside analogues on NK cell activity at lower doses paralleled effects on T-cytotoxic lymphocytes (Fig. 1; Table 1); however, at higher doses, T-cytotoxic lymphocytes appeared to be more sensitive to inhibition by the dAdo analogues than were NK cells (Fig. 1; Table 1). Kumagai et al. (34) reported an accessory function of NK cells for alloimmune CTL generation and that this function is mediated by interleu-
kin 2 and interferon produced by NK cells. Dosch et al. (55) and van den Akker et al. (56) showed that deoxyguanosine abrogates in vivo murine suppressor cell development, whereas T-helper function and B-cell function seem not to be affected. It has been reported that deoxyguanosine (57) and dAdo (53) are capable of inhibition of human T-suppressor but not T-helper cells.

The striking similarity between effects of DCF (i.e., Ratech et al.) (3) and Tub on primary antibody response in mice suggests that, at least in part, one aim of the current experiments may have been met. That is, this Ado analogue appears to mimic (with regard to humoral immune responses) the effects of the ADA inhibitor DCF. The observation that effects of the two dAdo analogues are diametrically opposed to those of Tub and DCF could be interpreted to suggest that some of the immune suppression observed after ADA inhibition (or absence) is mediated by Ado rather than dAdo.

In summary, the current observations indicate that purine nucleoside analogues can selectively modulate immune function. Specifically, Tub inhibited both T-lymphocytic and NK cell activities at doses that did not reduce primary antibody response, whereas the reverse was true for CldAAdo and FaraAAMP. Assuming the analogues mimic endogenous nucleosides, this result suggests that NK cells are more sensitive to Ado than are those cells responsible for primary antibody response, and the reverse is true for dAdo.

REFERENCES

12. Migliorati, G., Frati, L., Pastore, S., Bonmassar, E., and Riccardi, C. Increase of the ADA inhibitor DCF. The observation that effects of the two dAdo analogues are diametrically opposed to those of Tub and DCF could be interpreted to suggest that some of the immune suppression observed after ADA inhibition (or absence) is mediated by Ado rather than dAdo.

In summary, the current observations indicate that purine nucleoside analogues can selectively modulate immune function. Specifically, Tub inhibited both T-lymphocytic and NK cell activities at doses that did not reduce primary antibody response, whereas the reverse was true for CldAAdo and FaraAAMP. Assuming the analogues mimic endogenous nucleosides, this result suggests that NK cells are more sensitive to Ado than are those cells responsible for primary antibody response, and the reverse is true for dAdo.


Selective Modulation of Antibody Response and Natural Killer Cell Activity by Purine Nucleoside Analogues

Teresa Priebe, Osama Kandil, Melita Nakic, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/17/4799

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.