Synergistic Antitumor Effects of Interleukin 2 and the Monoclonal Lym-1 against Human Burkitt Lymphoma Cells in Vitro and in Vivo

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ABSTRACT

Interleukin 2 (IL-2) regulates immune responses by inducing proliferation and differentiation of T-cells into cytotoxic cells, inducing lymphokine activated killer activity and enhancing antibody dependent cellular cytotoxicity (ADCC). Lym-1, a monoclonal antibody, recognizes a membrane antigen present on the surface of B-lymphoma cells and can be used for ADCC. To study the efficacy of combination therapy with IL-2, lymphokine activated killer activity, and Lym-1. In vitro ADCC assays using Lym-1 showed that preincubation of peripheral blood lymphocytes with IL-2 had a synergistic antitumor effect. The maximum synergism was achieved when peripheral blood lymphocytes were incubated with IL-2 for 3 days as compared to 1 or 2 days, with the optimal concentration of IL-2 being 1000 units/ml. This effect was specific for Lym-1 as demonstrated by experiments using an irrelevant (antimalanoma) monoclonal antibody or an irrelevant target cell (A375). The ADCC was blocked by an anti-Fc receptor antibody (3G8). In vivo experiments performed by growing Raji tumors in nude mice also demonstrated the increase in ADCC and the synergism between IL-2 and Lym-1 in terms of decreased tumor size and growth. The mechanism of this synergy is probably from activation of cells mediating ADCC. This raises the possibility that treatment of patients with low doses of IL-2 in combination with Lym-1 may enhance immune responses and thereby antitumor activity.

INTRODUCTION

Interleukin 2 is a peptide secreted by lymphocytes that regulates immune responses. IL-2, on binding to IL-2 receptors on T-cells, induces proliferation and differentiation of T-cells. It has been shown that IL-2 can also potentiate the NK activity of PBL in vitro (1–3). Preliminary results of clinical trials with IL-2 (4–9) have shown that it is associated with major toxicity, most prominent of which is fluid retention resulting from a capillary permeability syndrome.

Recently, Epstein et al. (10) have developed a murine IgG2a MoAb Lym-1 that recognizes a membrane antigen present on the surface of malignant B-cells. Lym-1 has several advantages for monoclonal therapy. The antigen recognized by Lym-1 is not shed from the surface of lymphoma cells either in cell culture or in patients and does not undergo modulation. In vitro data demonstrate that Lym-1 can mediate ADCC and complement mediated cytotoxicity against lymphoma cells (11).

Miller et al. (12) have used monoclonal antiidiotype antibodies for the treatment of B-cell lymphoma. In their study there was a correlation between therapeutic outcome and number of host T-cells infiltrating the tumor. Thus, there may be host components that might be modulated for more optimum results with MoAb therapy.

A preliminary clinical trial using Lym-1 alone has been completed in 10 patients with refractory lymphoma at USC (11). In that trial, Lym-1 alone was not successful in causing antitumor responses. One reason may be inadequacy of the host effector cell responses similar to those mentioned above. Since IL-2 is immunomodulatory as mentioned above, we reasoned that prior administration of IL-2 may enhance the activity of host cells required for MoAb therapy. Thus, IL-2 and Lym-1 may be a potent combination. We therefore performed in vitro and in vivo studies to test this hypothesis.

MATERIALS AND METHODS

Tumor Cells. Raji cells, a Burkitt lymphoma cell line with binding sites for Lym-1, was kindly donated by Dr. Alan Epstein, USC School of Medicine. A375, a melanoma cell line from American Type Culture Collection, has no Lym-1 antigens on its surface.

Monoclonal Antibodies. Lym-1, (obtained from Dr. Alan Epstein) (10, 11), is an IgG2a murine MoAb which has B-cell specificity. A random screening of lymphoma biopsies has shown that the majority of B-cell tumors are Lym-1 positive. Using 125I-labeled preparations of purified Lym-1, it has been determined that there are also a relatively large number of binding sites for this antibody per tumor cell. The monoclonal antibody ZME against melanoma (gift of Hybritech Corp., San Diego, CA) was used as a positive control of ADCC against the melanoma A375. The antibody against Fc receptor (3G8) was kindly donated by Dr. Jay Unkeless of Mount Sinai Medical Center and Dr. Dixon Gray of USC Medical Center (13). The anti-B16 melanoma antibody was donated by the NIH (Bethesda, MD).

IL-2. Recombinant human IL-2 was kindly donated by Cetus (Emeryville, CA). The IL-2 had a specific activity of 3 x 10^6 units/mg. The purity of the IL-2 was determined to be 98% by sodium dodecyl sulfatedodecyl polyacrylamide gel electrophoresis and the endotoxin level was less than 0.01 ng/ml as determined by the Limulus amebocyte assay. PBL (1 x 10^8/ml) were incubated with IL-2 at varying concentrations (100, 1,000, or 10,000 units/ml, as stated in the experiment) for 1, 2, 3, or 7 days as indicated. The incubations were performed in tissue culture flasks in the presence of CM as described previously (7).

In Vitro ADCC Assay. 51Cr release assays (4 h) were performed as described in Ref. 11. Tumor target cells (Raji cells) were labeled with 100 μCi of 51Cr for 2 h in 0.5 ml of CM, then washed twice. Target:effector ratios used were 1:100, and 1:1. Raji cells (5 x 10^6) were added to each well of a 96-well round bottomed microtiter plate. Then PBL (5 x 10^6) activated as indicated in each experiment, were added to each well. Lym-1 (10 µl; 5.81 mg/ml), ZME, or 3G8 were added to one-half of the wells and the Skaton Titretek system and counted in a γ counter. Percentage of lysis was then calculated as:

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\% \text{ of lysis} = \frac{\text{Experimental cpm} - \text{spontaneous cpm}}{\text{Maximum cpm(tr)} - \text{spontaneous cpm}}
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where spontaneous cpm was measured by incubation of Raji cells with CM and maximum cpm was measured by incubation with 0.1 N HCl. Data are expressed as mean ± SE of triplicate determinations.

In Vivo Studies. Eight-week-old BALB/c nude mice were irradiated with 500 rads. Burkitt lymphoma cells (1 x 10^7 Raji cells) were injected i.m. in the thigh. Treatment with IL-2, Lym-1, or peripheral blood lymphocytes activated in IL-2 for 3 days (PBL) LAK was then started when palpable tumor was present (usually 10 days later). Lym-1, 150
RESULTS

Lym-1 Has ADCC Activity Specific for Raji Cells. Previous experiments have shown that Lym-1 has ADCC activity (11). We demonstrated the specificity of this in vitro antitumor ADCC activity by using an irrelevant cell line, A375 melanoma, which does not have any receptors for Lym-1, and the irrelevant monoclonal antibody ZME which is specific against the melanoma. As shown in Table 1, when Lym-1 is incubated with PBL and A375, no tumor lysis is seen. Similarly the antimelanoma MoAb ZME (which can mediate ADCC against melanoma cells) incubated with Raji cells and PBL fails to show any ADCC antitumor activity above that of PBL alone.

IL-2 Enhances ADCC Activity. In vitro 51Cr assays with labeled Raji cells were used to study the antitumor efficacy of Lym-1 alone, IL-2 activated (LAK) cells alone, and LAK cells in combination with Lym-1. We also used 3G8 which blocks the Fc receptor to check whether the synergistic activity of LAK and Lym-1 is via ADCC, and used an irrelevant ZME antibody (antibody against melanoma) to check for specificity of the ADCC. As shown in Table 2, Lym-1 alone had minimal antitumor activity PBL. LAK cells alone had a percentage of lysis of 22. However, by addition of Lym-1 to LAK cells (i.e., preincubation of PBL with IL-2), there was a significant enhancement in antitumor activity in our experiment (Table 2). This enhancement was abolished by the addition of 3G8 to the assay. Also ZME antibody did not lead to ADCC enhancement (Table 2). Similar results were obtained when this experiment was repeated.

Pharmacokinetics of Synergism with IL-2 and Enhancement of ADCC Activity. We studied the pharmacokinetics of the enhancement of Lym-1 ADCC activity by IL-2 by incubating PBL with IL-2 at varying concentrations (100, 1,000, or 10,000 units/ml). The optimal LAK activity was achieved at an IL-2 concentration of 1,000 units/ml (Table 3). We also studied varying durations of incubation (1, 2, and 3 days and, in a repeat of experiments, 1, 3, and 7 days). The optimal LAK activity was achieved when incubated for 3 days (Table 4).

In Vivo Antitumor Activity of PBL LAK, IL-2, and Lym-1.

Eight-week-old nude mice were given injections of Raji cells as described in “Materials and Methods” and were divided into 7 treatment groups as shown in Table 5. Tumor size was used as the evaluating parameter and results are as shown. Thus, again the mice receiving the Lym-1 plus IL-2 or LAK plus IL-2 treatments showed the greatest tumor regression. No tumor regression was observed with an irrelevant antibody (α B16 melanoma antibody was used) in combination with LAK plus IL-2. In a separate experiment, survival advantage was monitored and the best results were achieved with treatment with IL-2 plus Lym-1 or PBL LAK plus IL-2 plus Lym-1 over IL-2 alone, Lym-1, or PBL LAK alone (data not shown). The IL-2 given here can induce endogenous LAK cells in vivo.
of the HLA-Dr antigen (11). Interferon induced by IL-2 also induces the production of γ-interferon which then enhances Lym-1 binding sites. In future experiments we will investigate the nature of host cell infiltrates, the optimal timing of IL-2 and Lym-1 treatments, and the class I and class II antigens on tumor cells before and after IL-2 therapy.

On the basis of our data, we suggest that clinical trials be done to further explore the therapeutic potential of the enhancement of ADCC of Lym-1 in patients with lymphomas. This is especially desirable since as single agents, IL-2 and Lym-1 have been limited by toxicity (6) and inefficacy (11), respectively. At USC such a clinical trial using combination therapy with IL-2 and Lym-1 MoAb in resistant non-Hodgkin’s lymphomas is under way. On the basis of our preclinical data, the combination should be efficacious, and moreover the dose requirements to achieve such therapeutic effects could prove to be less than when these agents are used by themselves, thus decreasing the toxicity encountered.

REFERENCES


12. Miller, R. A., Maloney, D. G., Warneck, E. R., and Levy, R. Treatment of non-Hodgkin’s lymphoma with MoAb is an attractive and potentially selective approach minimizing toxicity. Clinical trials with the MoAb Lym-1 in the treatment of non-Hodgkin’s lymphoma have been done at USC (11). Lym-1 alone produced only minor antitumor responses. Analysis of treatment variables has demonstrated that a major limitation is the inadequacy of host effector cell response to the monoclonal serotherapy. This confirms observations made by Miller et al., (12) using antiidiotype antibody therapy, where host-tumor interaction was a major variable in response. As shown in our experiments, Lym-1, when used in combination with IL-2, was successful in preventing the progression of human tumor in nude mice. These findings are similar to those reported by Miller et al., using antiidiotype MoAb. Our results are potentially more advantageous because they involve a general monoclonal antibody rather than an antiidiotype, the generation of the latter requiring a tedious process.


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