Absence of Macrophage Involvement in the Passive Serum Therapy of Friend Leukemia Virus-induced Disease

Jeffrey J. Collins and Ralph Snyderman

Departments of Microbiology and Immunology, Surgery [J. J. C.], and Medicine [R. S.], Duke University Medical School, Durham, North Carolina 27710

ABSTRACT

The possible involvement of host macrophages in the passive serum therapy of Friend leukemia virus (FLV)-induced disease has been examined with the use of agents inhibiting normal macrophage functions, including silica and a tumor-produced macrophage chemotaxis inhibitor. Under conditions in which macrophage functions are at least transiently abrogated by these agents, no effect was seen on the anti-FLV protection afforded by the passive administration of chimpanzee anti-FLV antiserum to infected DBA/2 mice, as monitored by the development of virus-induced splenomegaly and the level of infectious virus. The macrophage inhibitors also did not influence the appearance of the host antiviral humoral immune response which normally accompanies serum protection. These results suggest that the normal functioning of host macrophages do not play a central role in the passive serum therapy protective mechanism leading to resistance to FLV infection.

INTRODUCTION

We have reported previously that mice challenged with a leukemogenic dose of FLV can be protected against the development of disease by subsequent passive therapy with heterologous antiserum raised against disrupted virus or the purified major viral envelope glycoprotein with a molecular weight of 71,000 (4, 12). A close correlation was shown to exist between protection by heterologous immune serum and the development of host (mouse) antiviral humoral immunity, with both of these parameters being inversely related to serum protection. Our current efforts are aimed at determining the precise mechanism by which passively administered heterologous serum leads to resistance against virus-induced disease, particularly the identification of those components of the host’s immune system which are involved in protection. An immune mechanism which would fit well with many of the features of the FLV serum therapy model system is antibody-dependent cellular cytotoxicity. Since macrophages have been reported to function as antibody-dependent cellular cytotoxicity effector cells, including in vivo tumor rejection model systems (5), and considering the increasing body of evidence that suggest macrophages play an important role in the immunological rejection of tumors (1), it was decided to examine the possible involvement of these cells in the serum protective process. Treatment of animals with various agents, including silica and carrageenan, which diminish the functional capacities of macrophages in vitro (2, 3, 8) have also been reported to abrogate macrophage functions in vivo (9, 10, 18). We have thus attempted to assess the role of macrophages in the passive serum therapy of FLV infection by administering serum to mice treated with silica. In addition, a previously described (11, 14, 16) inhibitor of macrophage chemotaxis produced by tumor cells was also utilized to examine the involvement of macrophages in the serum-mediated induction of resistance to FLV. We report experiments which indicate that agents which interfere with the normal functioning of host macrophages do not adversely affect the successful passive therapy of FLV infection following administration of heterologous anti-FLV antiserum.

MATERIALS AND METHODS

Virus

Preparation of stock FLV was as described (4). Briefly, the spleens of FLV-infected DBA/2 mice (Microbiological Associates, Inc., Bethesda, Md.) were collected when they became grossly palpable (usually 2 to 3 weeks after infection, at which time they weighed >900 mg each) and used to prepare a 20% (w/v) preparation in Roswell Park Memorial Institute Tissue Culture Medium 1640 by homogenization for 30 sec and clarification by centrifugation at 2000 x g for 15 min. The supernatant was removed and filtered successively through 1.2- and 0.8-μm pore size Millipore filters, and the cell-free filtrate was stored in small aliquots at -70°C.

Macrophage Chemotaxis Inhibitor

The tumor-produced macrophage chemotaxis inhibitor was prepared as described previously (11, 14). Briefly, C3H mouse 129 hepatoma cells were suspended in phosphate-buffered saline (0.15 μ NaCl, 0.1 μ phosphate, pH 7.2) to a concentration of 5 x 10⁶ cells/ml, and the cells were lysed by 2 cycles of sonication (Branson; Danbury, Conn.) for 30 sec each followed by centrifugation for 10 min at 1800 x g. The low-molecular-weight fraction was obtained by overnight dialysis of 1 volume of clarified supernatant against 2 volumes of Roswell Park Memorial Institute Tissue Culture Medium 1640, pH 7.0, followed by ultrafiltration with an Amicon Centriflo membrane cone (Amicon Corp., Lexington, Mass.) to collect the material with molecular weight of <25,000. The efficacy of this preparation was monitored by the in vitro macrophage chemotaxis inhibition assay described previously (11, 14).

Treatment with Macrophage Inhibitors

Silica. Treatment of mice with silica was performed as de-
scribed (19). Briefly, silica particles of 0.25 μm average size (kindly provided by Dr. Stephen Russell, University of North Carolina Medical School, Chapel Hill, N. C.) were suspended in sterile isotonic saline at 10 mg/ml and dispersed by sonication for 1 min just prior to inoculation. DBA/2 mice were inoculated i.v. in the tail vein with 0.3 ml of the silica suspension; the mice thus received a dose of 3 mg silica/inoculation. The timing of silica treatment relative to FLV and serum inoculation is indicated in "Results."

Chemotaxis Inhibitor

Treatment of DBA/2 mice with the tumor-produced macrophage chemotaxis inhibitor consisted of i.p. inoculation of 0.2 ml of the ultrafiltrate preparation (described above) at a dilution (1:25) which provided maximal inhibitory activity in previous tests. See "Results" for the different treatment schedules used relative to the FLV and serum inoculations. Control mice received 0.2 ml of MEM i.p. at the same times.

Passive Serum Therapy

Preparation of the chimpanzee anti-FLV antiserum raised against purified Tween:ether-disrupted FLV has been described in detail (4). Virus challenge consisted of i.p. inoculation of 0.2 ml of a 10⁻² dilution of the stock FLV preparation, and serum therapy involved 4 i.p. inoculations of 0.2 ml each of normal or the anti-FLV chimpanzee serum 3, 6, 9, and 12 days after FLV.

Serological Assays

RIA. The double antibody RIA procedure described by Strand and August (18) was used with purified FLV glycoprotein with a molecular weight of 71,000 labeled with ¹²⁵I according to the method of Greenwood et al. (5). The specific activity of the labeled antigen ranged from 5 x 10⁹ to 2 x 10¹⁰ cpm/μg protein. Between 1 and 5 ng of ¹²⁵I-labeled FLV glycoprotein with a molecular weight of 71,000 in a 10-μl volume was used in the RIA. Normal mouse serum (30 μl) and test serum (10 μl) were added to the labeled antigen and incubated at 37° for 3 hr and at 4° overnight. All dilutions were made in RIA buffer (20 mM Tris-HCl, pH 7.5:100 mM NaCl:1 mM EDTA containing 2 mg/ml bovine serum albumin). Rabbit anti-mouse immunoglobulin antiserum (30 μl) (Cappel Laboratories, Cochranville, Pa.) was then added and incubated for 1 hr at 37° and for 3 hr at 4°. Immunoprecipitates were collected by centrifugation at 8000 x g for 2 min, washed twice with RIA buffer (without bovine serum albumin), and counted in a Packard Autogamma Counter (Packard Instrument Co., Downers Grove, Ill.).

Cytotoxicity. The [¹⁴C]nicotinamide release microcytotoxicity assay using FLV-producing cells of the Eveline STU mouse cell line as targets has been described in detail (4). Briefly, 2 x 10⁴ Eveline cells were seeded in each well of a Falcon microtest plate (No. 3034; Oxnard, Calif.) in 10 μl MEM supplemented with 10% fetal calf serum and 25 μCi/ml of [¹⁴C]nicotinamide (60 μCi/mmol; Amersham/Searle, Arlington Heights, Ill.). After 24 hr of growth, when the cells had just reached confluence, the plates were washed 3 times with cold MEM (without serum), wash fluid was aspirated from the plates and wells, and 5 μl of the appropriate test serum dilution were added to triplicate wells. The plates were incubated for 15 min at 37° in a CO₂ incubator and then 10-μl rabbit complement (Pel Freeze; Rogers, Ark.) diluted 1:6 was added to appropriate wells. After a further 15-min incubation at 37° in a CO₂ incubator, 5 μl were removed from each well for counting in a Beckman LS-350 β-irradiation counter (Beckman Instruments, Palo Alto, Calif.).

Maximum release of radioactivity was determined by the addition of 15 μl/well of 0.5% Triton X-100 at the start of the first incubation. Results are expressed as percentage of SR, where:

\[
\% \text{SR} = \frac{\text{test cpm} - \text{background cpm}}{\text{maximum release cpm} - \text{background cpm}}
\]

Background is either complement control or medium control, and a SR ≥20% reflects significant cell lysis.

RESULTS

Given the fact that serum therapy must be started within the first week after FLV challenge to be effective (4, 13) and that the inhibitory effect of i.v.-inoculated silica on macrophage function is most marked for a period of approximately 3 days (9, 19), it was decided to treat the mice with silica on Day 2, i.e., 2 days after virus challenge and 1 day before the first serum treatment. The results (Table 1) demonstrate that whereas mice receiving either no serum or normal chimpanzee serum all developed splenomegaly by Day 22 after FLV challenge, mice receiving the standard treatment with chimpanzee anti-FLV serum were nearly uniformly protected against virus-induced splenomegaly. Most importantly, no difference was seen between mice treated with the same chimpanzee serum (normal or immune) regardless of whether they had received silica or not, both in terms of splenomegaly induction and spleen tissue infectious virus content (Table 1). Furthermore, the usual correlation (4) between protection and the development of a host antiviral humoral immune response, as measured in cytotoxicity testing on FLV-infected mouse cells and in RIA versus ¹²⁵I-labeled FLV gp71, was also seen in both the silica-treated and nontreated mice (Table 1; Chart 1).

In an attempt to confirm the results obtained by silica treatment which suggested a lack of involvement of macrophages in the resistance to FLV-induced disease by passive therapy with the chimpanzee anti-FLV serum, the second approach using the previously described tumor-produced macrophage chemotaxis inhibitor (11, 14, 16) was utilized. Because of the transient nature of this factor’s effect in vivo (11, 14) as well as the fact that mice become refractory to it after multiple treatments (5) it was decided to examine a number of different treatment schedules including 2 doses given 2 days before and 2 days after virus, 2 doses on Days 2 and 8 after virus, and 4 doses on Days 2, 5, 8, and 11 after virus (i.e., 1 day before each serum inoculation). The efficacy of the inhibitor was monitored in vitro by assaying unstimulated peritoneal cells for chemotactic and phagocytic activity as described previously (11, 14). As with the silica treatment, none of the chemotaxis inhibitor treatments interfered with the ability of the chimpanzee anti-FLV serum to protect mice against FLV infection (Table 2). However, only in the case of the protocol used in Experiment

\[ M. C. Pike and R. Snyderman, unpublished observations. \]
The time the mice were sacrificed (data not shown), although, chemotaxis was still suppressed by the inhibitor treatment at 3 (Days 2 and 8) could it clearly been shown that macrophage was again observed, regardless of whether the mice received the chemotaxis inhibitor or not (Table 2; Chart 2).

DISCUSSION

While the results presented in this report strongly suggest that macrophages do not play a central role in the passive serum therapy protective mechanism leading to resistance to FLV infection, this conclusion must be tempered by the uncertainties inherent in treating mice with agents such as silica, carrageenan, or the chemotaxis inhibitor (7). Important questions exist as to the specificity of these agents for macrophages, the duration of their effect, the most appropriate time of treatment with them relative to the serum therapy protocol, as well as the degree of their effectiveness under the conditions of the present experiments. Nevertheless, since no effect of silica treatment on serum therapy was seen, the possible influence of this agent on immune cells other than macrophages is not relevant to the present study.

While we could confirm a suppressive effect of the tumor-produced inhibitor given on Days 2 and 8 on macrophage chemotaxis that was measured on Day 15 utilizing peritoneal cells and the in vitro chemotaxis assay described previously (11, 14), attempts to establish decreased functions with the silica treatment and the other chemotaxis inhibitor treatment schedules used were equivocal. Since the effects of these agents are of limited duration (9, 11, 14, 19), it is not surprising that assays of macrophage activity 7 or more days after the last treatment yielded inconclusive results. However, we have confirmed in uninfected DBA/2 mice that macrophage functions measured by chemotaxis (11, 14) and the phagocytosis of 51Cr-labeled sheep RBC opsonized with rabbit IgG (17), are suppressed for approximately 3 days after i.v. inoculation of 3 mg silica per mouse,6 and others have demonstrated that identical treatment protocols with silica or the chemotaxis inhibitor strongly suppress macrophage activities (9, 11, 14, 16).
Chart 1. Direct correlation between protection against the development of FLV-induced splenomegaly and the appearance of a mouse antiviral immune response, as determined by serum cytotoxicity on FLV-infected mouse cells and in RIA versus 125I-labeled glycoprotein with a molecular weight of 71,000. •, individual serum sample collected on Day 22 with sera included from all groups presented in Table 1 except uninfected control mice of group 6. Serum cytotoxic reactivity expressed on a scale of + to +++++, where + = 20 to 40% SR, ++ = 40 to 60%, +++ = 60 to 80%, and ++++ >80% SR based on complement control. Serum RIA reactivity expressed as degree of precipitation of 125I-labeled FLV glycoprotein with a molecular weight of 71,000 by serum at a 1/5 dilution on a scale of + to +++++, where + = 10 to 15% precipitation of input label, ++ = 15 to 20%, +++ = 20 to 25%, and ++++ >25% (mouse sera are much weaker than hyperimmune anti-FLV or anti-FLV glycoprotein with a molecular weight of 71,000 sera and rarely give 50% precipitation of input label). Spleen weight ≥250 mg is considered to represent significant splenomegaly.

16, 19). Furthermore, given the fact that mice receiving multiple inoculations of the chemotaxis inhibitor become refractory to its effect,5 the failure to observe clear-cut abrogation of macrophage chemotaxis with peritoneal cells obtained from mice of Groups 1 and 3 (see Table 2) 4 days after the fourth injection is not unexpected.

In summary, notwithstanding the limitations inherent in the use of agents interfering with normal macrophage functions, the present results clearly suggest that macrophages do not play a critical role in the passive serum therapy of FLV-induced disease nor in the generation of a humoral response by protected mice to viral antigens such as gp71. While it could be argued that the transient effect of the inhibitory agents used might allow a functional role for macrophages later in the serum protective process, the requirement that serum therapy be initiated within 7 days after FLV infection if protection is to be achieved (4, 13) suggests that the essential elements in the passive therapy mechanism must be activated early and renders such an argument less probable. In fact, it was the consideration of the importance of beginning the serum treatments during the early stages of the infection process that dictated our choice of the inhibitor treatment schedules used. Furthermore, in accordance with the present conclusion that macrophages do not play a central role in the serum protective process, although by no means eliminating any contribution by this immune cell population, adoptive cell transfer experiments to be reported elsewhere7 suggest that non-T lymphocytes may be the essential component of the host immune system involved in successful serum therapy.

7 J. J. Collins, E. V. Genovesi, and F. Santilippo, manuscript in preparation.
Chart 2. Direct correlation between protection against the development of FLV-induced splenomegaly and the appearance of a mouse antiviral immune response, as determined by serum cytotoxicity of FLV-infected mouse cells and RIA versus $^{125}$I-FLV glycoprotein with a molecular weight of 71,000. ●, individual serum sample collected on Day 15 with sera included from all groups presented in Table 2. See legend to Chart 1 for additional description.

ACKNOWLEDGMENTS

We thank L. Bolio, D. Livnat, M. McLean, D. Sackie, and A. Hartman for excellent technical assistance and Dr. M. Venkataraman for assistance with the i.v. inoculations of silica.

REFERENCES


Absence of Macrophage Involvement in the Passive Serum Therapy of Friend Leukemia Virus-induced Disease

Jeffrey J. Collins and Ralph Snyderman


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/3/557

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.