woodite, are typical of crystals annealed at high temperatures, whereas neighboring untransformed olivine has a high density of dislocations corresponding to the (110)[001] slip system typical of shocked crystals (*17*); this is to be expected since the high-pressure phases grew in replacement of shocked minerals. The positive identification of ringwoodite and majorite in a shocked meteorite supports the view that it is possible to synthesize high-pressure silicate phases by shock wave experiments.

Although our observations on ringwoodite are still fragmentary, they all point to a remarkable similarity to other spinels; we find the same stacking faults and the same slip systems as in $MgAl_2O_4$ spinel, for instance. It seems reasonable to think that many other similarities will eventually be found; thus we expect to find straight edge dislocations with an $a/4$ [110] Burgers vector, dissociated in their climb plane (110) with the least energetic fault (*16, 18*). If this is the case, we may venture to draw a few tentative conclusions regarding the rheology of the mantle transition zone (*19*): intracrystalline flow of γ spinel may proceed by slip on (111) planes (probably difficult and controlled by pinching of the stacking fault which does not lie in the glide plane) or by pure climb of edge dislocations dissociated in their climb planes (*17, 18*). If the grain size of spinel is small in the mantle (*19*), it is possible to consider grain-boundary sliding accommodated by Nabarro or Coble creep in the grains as an important flow mechanism (*20*); this would make it possible to think that the mantle viscosity does not increase much in the transition zone (*21*) and that there is no rheological barrier to convection below 400 km (*22*).

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9. R. Jeanloz [*Proc. 10th Lunar Planet. Sci. Conf.* (1979), p. 619] reported that he could find no spectroscopic evidence for ringwoodite in the purple grains of Catherwood, whereas majorite could be identified. He tentatively attributes this to the fact that ringwoodite is intimately mixed with abundant majorite (R. Jeanloz, personal communication).
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20. This is the so-called superplastic creep. P. J. Vaughan [*Eos* **59**, 1185 (1978); personal communication] experimentally found that Mg_2GeO_4 spinel deformed by this mechanism.
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Mouse Leukemia: Therapy with Monoclonal Antibodies Against a Thymus Differentiation Antigen

Abstract. *Monoclonal antibodies against a thymus cell differentiation antigen (Thy-1.1) were effective in the therapy of a transplanted mouse leukemia. Passive immunization resulted in high titers of cytotoxic antibody in the serum of treated mice and the suppression of metastatic tumor cells. The tumor-suppressive effects of the monoclonal antibodies were amplified by the administration of exogenous complement. This combined antibody and complement therapy resulted in the cure of leukemia in a significant proportion of the treated animals.*

The treatment of malignant disease with antibody has been of considerable interest to oncologists and immunologists throughout the past decade (*1*). Although inhibition of tumor growth has been demonstrated in experimental systems by the passive administration of antibody, these effects were usually found to be minimal, short-lived, or therapeutic against only small tumors. Solid tumors were relatively resistant to antibody therapy, whereas tumors of lymphoid origin were found more responsive. One of the limiting factors in the success of antibody therapy has been the lack of sufficiently high-titered antibodies of defined class, avidity, and specificity. In fact, the overall difficulties associated with these technical aspects have tended to dampen the enthusiasm for a more generalized approach to the serotherapy of cancer.

A reappraisal of the role of serotherapy in the treatment of tumors may now be warranted. With recent advances in somatic cell hybridization techniques (*2*) it is now possible to obtain permanent cell lines that continuously produce monoclonal antibodies of defined specificity. This is accomplished by the hybridization of B lymphocytes from immunized donors with myeloma cells in culture (*2*). Antibodies obtained from these hybrid cell lines are chemically homogeneous, react with constant avidity to single antigenic determinants, and can be isolated in quantities previously not obtainable by conventional methods. In this study we demonstrate that monoclonal antibodies directed against a T cell differentiation antigen (Thy-1.1) dramatically inhibit the growth of a transplantable AKR mouse lymphoma.

Mice of the AKR, 129, and C57BL/6 (B6) strains were purchased from the Jackson Laboratory. F_1 hybrids [(129 × AKR)F_1 and (B6 × AKR)F_1] prepared between these strains were bred in our mouse colony. The AKR SL2 leukemia (used for serotherapy) was derived from a spontaneous thymoma (*3*); SL2 cells passaged both in vivo and in vitro expressed high concentrations of Thy-1.1 antigen on the cell surfaces.

Hybrid cells producing monoclonal antibodies against the Thy-1.1 antigen were isolated from a fusion performed with BALB/c MOPC21 NSI/1 myeloma

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cells and the lymphocytes of 129 strain mice that were immunized with allogeneic AKR SL3 leukemia cells. Details of the procedures used to select and clone hybrid cells have been described (*4*). The antibody products of the hybrid cells were assayed by an antibody-binding assay with ^{125}I-labeled protein A from *Staphylococcus aureus* (*4*). For these assays we used cell membrane fragments that were exfoliated by AKR SL3 cells into the culture medium as a source of Thy-1.1 antigen. Cells from each of the cloned cell lines were then inoculated intraperitoneally in syngeneic (129 × BALB)F_1 mice for the production of hybridomas and ascites fluids that contained high-titered antibodies (*4*).

Monoclonal antibodies from two independent cell lines were assessed in this study. Both antibodies, 19-A10 and 19-E12, showed specificity for the Thy-1.1 antigen in cytotoxic assays (*5*). In immune precipitation assays with Nonidet P-40 lysates of ^{125}I-surface labeled AKR SL2 cells these antibodies precipitated a single protein of 27,000 daltons (*6*). Both antibodies were of the immunoglobulin (Ig)G_{2a} subclass, and in two-dimensional polyacrylamide gel electrophoresis they showed homogeneity indicative of monoclonal products. Ascites fluids from hybridoma-bearing mice contained 21 and 39 mg/ml, respectively, of antibodies 19-A10 and 19-E12. In preliminary tests the ascites fluids were tested in complement (C)-dependent cytotoxicity and antibody-dependent cell cytotoxicity (ADCC) assays with ^{51}Cr-labeled AKR SL2 cells. The ascites fluid 19-A10 had a titer of 5×10^{-5} in C-dependent cytotoxicity assays (50 percent lysis) and 1.25×10^{-5} in ADCC assays (20 percent lysis), while the ascites fluid 19-E12 had a titer of 8×10^{-6} in C-dependent cytotoxicity and 2×10^{-6} in ADCC.

In the first experiments, 19-A10 antibody was examined for its activity in

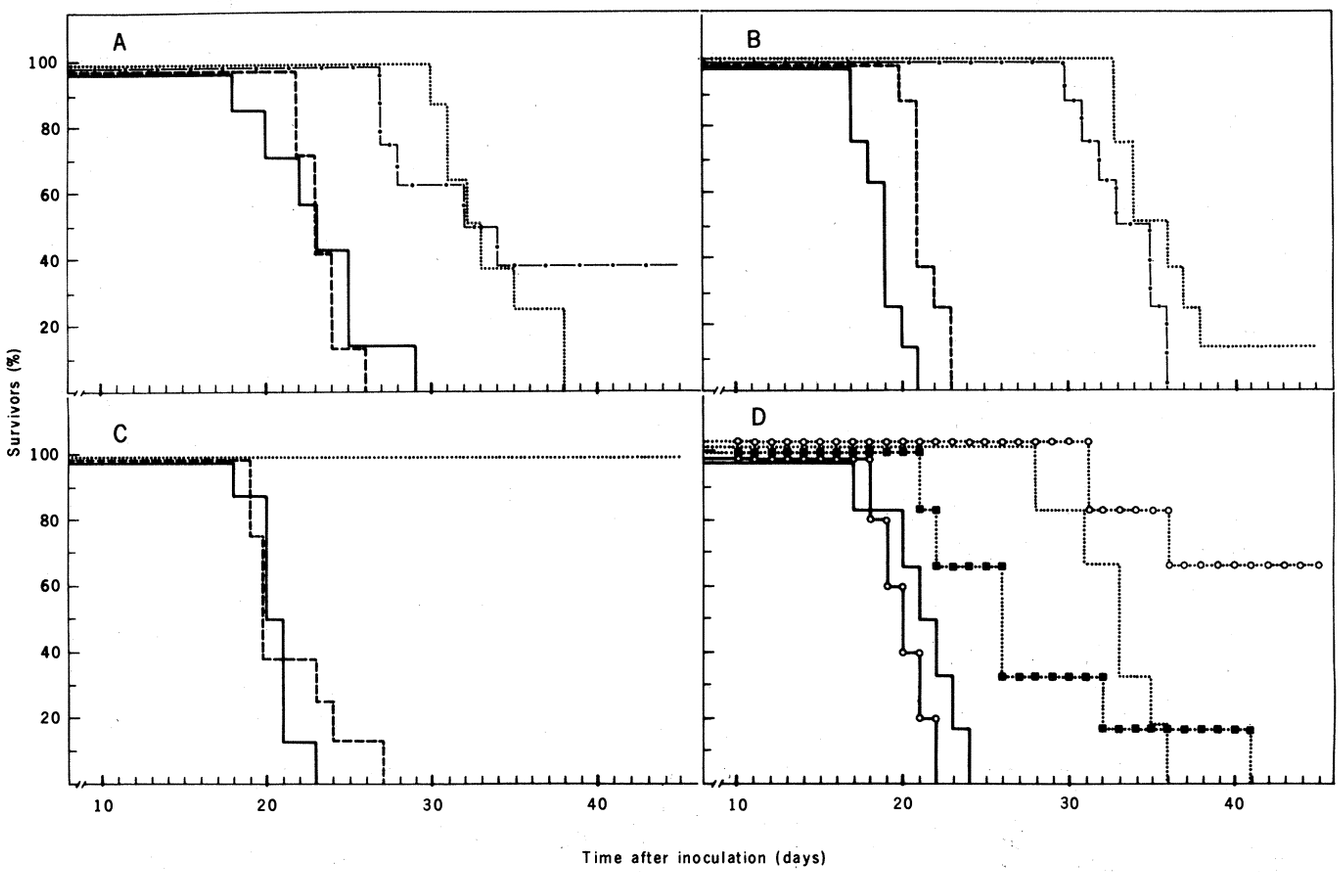

Fig. 1. Serotherapy of AKR SL2 leukemia with monoclonal antibodies to Thy-1.1. (A) Four groups of (129 × AKR)F_1 hybrid mice were inoculated subcutaneously with 10^6 AKR SL2 cells on day 0. Group 1 (solid line), seven untreated control mice; group 2 (broken line), seven control mice treated with rabbit serum (as a source of C) alone; group 3 (dotted line), eight test mice treated with 19-A10 ascites fluid alone; group 4 (dots and dashes), eight test mice treated with 19-A10 ascites fluid and rabbit serum. Treatments were performed as follows: day 0, 100 μl of 19-A10 ascites fluid or 100 μl of rabbit serum, or both, were injected intravenously; days 2, 4, and 7, 50 μl of 19-A10 ascites fluid or 100 μl of rabbit serum, or both, were injected intraperitoneally. (B) Four groups of (B6 × AKR)F_1 hybrid mice were each inoculated subcutaneously with 3×10^6 AKR SL2 cells on day 0. Group 1 (solid line), eight untreated control mice; group 2 (broken line), eight control mice treated with rabbit serum alone; group 3 (dots and dashes), eight test mice treated with 19-A10 ascites fluid and rabbit serum; group 4 (dotted line), eight test mice treated with 19-E12 ascites fluid and rabbit serum. Treatments were performed as follows: day 0, 100 μl of ascites fluid or 100 μl of rabbit serum, or both, were injected intravenously; days 3, 7, 10 and 14, 50 μl of ascites fluid or 100 μl of rabbit serum, or both, were injected intraperitoneally. (C) Three groups of AKR mice were each inoculated subcutaneously with 10^5 AKR SL2 cells on day 0. Group 1 (solid line), eight untreated control mice; group 2 (broken line), eight control mice treated with rabbit serum alone; group 3 (dotted line), six test mice treated with 19-A10 ascites fluid and rabbit serum. Treatments were performed as follows: day 0, 100 μl of 19-A10 ascites fluid or 100 μl of rabbit serum, or both, were injected intravenously; days 3, 7, 10 and 14, 50 μl of 19-A10 ascites fluid or 100 μl of rabbit serum, or both, were injected intraperitoneally. (D) Five groups of (B6 × AKR)F_1 hybrid mice were each inoculated subcutaneously with 3×10^6 AKR SL2 cells on day 0. The local subcutaneous tumors were removed surgically from mice in groups 2, 4, and 5 on day 10. Group 1 (solid line), six untreated control mice; group 2 (solid line and circles), five untreated control mice, surgery on day 10; group 3 (dotted line), six test mice treated on days 0, 3, 5, and 7 with 19-A10 ascites and rabbit serum; group 4 (dotted line and circles), six test mice treated on days 0, 3, 5, and 7 with 19-A10 ascites and rabbit serum, surgery on day 10; group 5 (dotted line and squares), seven test mice treated only on day 7 with 19-A10 ascites and rabbit serum, surgery on day 10. Treatment on day 0 was 50 μl of 19-A10 ascites and 100 μl of rabbit serum administered intravenously; other treatments on days 3, 5, and 7 were the same dose administered intraperitoneally.

vivo against a transplant of AKR SL2 cells. Since serum samples from mice of the AKR strain were known to contain low concentrations of the complement component C5, the therapy studies were initially performed in F_1 hybrid mice with high concentrations of serum C5 (*7*). For this purpose (129 × AKR)F_1 hybrid mice were each inoculated subcutaneously with 10^6 AKR SL2 cells [a dose 2 logs greater than the 100 percent lethal dose (LD_{100})]. The mice were then separated into four groups for treatment, which was initiated 1 to 2 hours after tumor inoculation. Mice in one test group were each treated with 19-A10 ascites fluid; mice in a second test group were each injected with 19-A10 ascites fluid and rabbit serum as a source of exogenous C. Two additional groups in this experiment were used as controls; one was untreated, while the other received rabbit serum alone. As shown in Fig. 1A, the leukemias grew progressively in the untreated mice and were uniformly lethal. The leukemia transplant was first observed as a local tumor at the injection site. By the time the local tumors had reached a size of 30 to 50 mm^2 (average radius squared), they had already metastasized and induced apparent systemic disease. The median survival time for both the untreated mice and mice receiving rabbit serum alone was 23 days. In contrast, mice treated with the 19-A10 ascites fluid showed prolonged survival, with a median of 33 days. This prolongation in survival was manifested primarily as a delay in the onset of systemic disease, not in retardation of tumor growth at the inoculum site. In fact, in the absence of gross systemic disease the local subcutaneous tumors in these mice grew to two to three times the size of the tumors in the untreated or rabbit serum–treated controls. A significantly greater therapeutic effect was achieved when the 19-A10 ascites fluid was administered with rabbit serum as an exogenous C source. In this case, three of the eight animals treated with 19-A10 ascites and rabbit serum did not develop either local or systemic disease. The median survival of the remaining five mice in this group that succumbed to leukemia was 33 days.

Assessment of serum antibody in treated mice showed that high titers (approximately 2.5×10^{-4}) of cytotoxic antibodies were achieved in vivo 24 hours after the last antibody injection. Since it was anticipated that the Thy-1.1 antibodies would react with the normal T cells of the host, it was of interest to note that the combined use of high-titered antibodies and rabbit serum did not cause obvious side effects or generalized toxicity. After cessation of treatment the titers of cytotoxic antibody in the serum dropped; at 2 weeks after the last injection the titers were at background levels ($< 10^{-2}$).

In the next experiment, the ascites fluids from both the 19-A10 and 19-E12 hybridomas were tested for antitumor activity in (B6 × AKR)F_1 hybrid and AKR parental mice that were inoculated with AKR SL2 cells. As shown in Fig. 1B, (B6 × AKR)F_1 hybrid mice were each inoculated subcutaneously with 3×10^6 AKR SL2 cells. Treatment with ascites fluids (in combination with rabbit serum) was initiated on the same day and then continued every third day for 2 weeks. The results again showed that the combined use of antibody to Thy-1.1 and exogenous C significantly prolonged survival. The median survival times for the untreated and rabbit serum–treated controls were 19 and 21 days, respectively; the median survival times for the groups treated with 19-A10 and 19-E12 ascites were 33 and 35 days. One animal treated with 19-E12 ascites fluid and rabbit serum showed neither local nor systemic leukemia. As observed before, the antibody-treated mice developed local subcutaneous tumors that grew to approximately three times the size of the control tumors before evidence of systemic disease appeared. In the second part of this experiment the 19-A10 ascites fluid (in combination with rabbit serum) was used to treat AKR SL2 that was carried as a transplant (10^5 cells injected subcutaneously) in AKR mice. As shown in Fig. 1C, all of the AKR mice that received ascites fluid and rabbit serum were cured of both the local tumor and systemic disease. In contrast, the untreated and rabbit serum–treated controls showed progressive tumor growth with mean survival times of 21 and 22 days, respectively.

From these studies it was concluded that the monoclonal antibody to Thy-1.1 prolonged survival by retarding the development of systemic leukemia. It was further noted that antibody alone had only limited effectiveness on local tumor growth. Presumably, the local tumor served as a reservoir for cells that could subsequently metastasize and kill the host when effective antibody titers were no longer present. This suggested a treatment regimen that included (i) the use of surgery to remove the local tumor and (ii) the use of passively administered antibody to eradicate metastatic tumor cells. Accordingly (B6 × AKR)F_1 hybrid mice were each inoculated subcutaneously with 3×10^6 AKR SL2 cells. The mice were then separated into five groups for treatment. Mice in two test groups were treated on days 0, 3, 5, and 7 with 19-A10 ascites fluid and rabbit serum. Mice in a third test group were treated with a single injection of 19-A10 ascites fluid and rabbit serum on day 7. Mice in the two control groups were not treated with either ascites fluid or rabbit serum. On day 10 the local subcutaneous tumors were surgically excised from mice of one control and two test groups (see legend to Fig. 1D) and the survival patterns were noted. As shown in Fig. 1D, the subcutaneous tumors had already metastasized to peripheral sites at the time of surgery, since all of the control mice, whether or not their tumors were excised, succumbed to systemic leukemia with a median survival of 20 to 21 days. In contrast, treatment of the mice with multiple doses of 19-A10 ascites fluid, rabbit serum, and surgery proved to be highly beneficial. Four of the six mice in the group that received multiple antibody treatments and surgery were cured of local and metastatic disease. Even mice treated with a single dose of 19-A10 ascites fluid and rabbit serum showed prolonged survival (median 28 days). Also shown in Fig. 1D is that mice treated with 19-A10 ascites fluid and rabbit serum alone had a prolonged survival compared to the untreated controls; although eventually, in the absence of surgery to remove the local tumor, each of these mice also succumbed to leukemia. In this case the median survival for mice treated with 19-A10 ascites fluid and rabbit serum was 33 days.

These studies demonstrate the utility of monoclonal antibodies for the treatment of leukemia cells. In some instances these antibodies were capable of curing mice of tumor transplant doses 100-fold greater than the LD_{100}. In other instances, through combined treatment with surgery, these antibodies were capable of curing established metastatic leukemias. As a generalization, antibody alone appeared to be more effective against systemic disease than against local tumor growth; inhibition of the latter required the additional administration of exogenous C. Whether the mechanism of action for the elimination of tumor metastases differed from the elimination of tumor at the inoculum site could not be determined from these studies. The most straightforward explanation for our results is that the monoclonal antibodies, in combination with C or ADCC mechanisms, directly killed the tumor cells at the local and metastatic sites. However, alternative explanations can also be considered. For example, it is conceivable

that the antibodies to Thy-1.1 exerted some of their protective effects by modifying the T cell system (for example, by elimination of suppressor cells) of the host.

The effectiveness of antibodies against differentiation antigens for the treatment of lymphoid tumors is of considerable clinical importance, because human leukemias also are known to contain differentiation antigens on the cell surface that are analogous to those described in mouse tumors (*8*). The results described here should add impetus to the development of cell lines that produce monoclonal human antibodies against similar types of antigens on human tumor cells.

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Hybridoma Produces Protective Antibodies Directed Against the Sporozoite Stage of Malaria Parasite

Abstract. *Hybrid cells secreting antibodies against sporozoites of* Plasmodium berghei *were obtained by fusion of plasmacytoma cells with immune murine spleen cells. The monoclonal antibodies bound to a protein with an apparent molecular weight of 44,000 (Pb44), which envelopes the surface membrane of sporozoites. Incubation of sporozoites in vitro with antibodies to Pb44 abolished their infectivity.*

Studies on immunity induced by the sporozoite stage of mammalian malaria, carried out in rodents, rhesus monkeys, and man, have shown that total protection can be achieved by repeated bites of infected mosquitoes or by intravenous injections of x-irradiated sporozoites (*1*). Antibodies to sporozoites also occur in the serum of more than 90 percent of the adults living in an area of hyperendemic malaria where they are frequently exposed to the bites of *Plasmodium falciparum*-infected mosquitoes (*2*).

The antigens involved in these immune responses, which are strictly stage-specific, have not been characterized. Rodents immunized with sporozoites produce antibodies that react in vitro with the membrane of the parasite, as demonstrated by circumsporozoite precipitation (CSP) (*3*), indirect immunofluorescence (*4*), and sporozoite neutralizing activity (*1*). Stage-specific antigens on the surface of rodent, simian, and human malaria sporozoites have also been found by ultrastructural observations (*5*) and serological tests (*4*). In experiments aimed at the characterization of the surface antigens of sporozoites of *P. berghei*, a parasite of rodent malaria, a membrane component with molecular weight close to 44,000 (Pb44) was specifically immunoprecipitated by the serum of vaccinated mice (*6*).

Here we report that we have hybridized sporozoite antibody-producing murine spleen cells with plasmacytoma cells; the hybrid cells produce antibodies to Pb44 that mediate the CSP and immunofluorescence reactions and have sporozoite neutralizing activity.

Mice of strain BALB/c, immunized by exposure to repeated bites of γ-irradiated *P. berghei*-infected mosquitoes, served as the source of spleen cells. Hybridization was carried out according to the method of Köhler and Milstein (*7*), with 1.4×10^7 plasmacytoma P3U1 cells (κ chain secretor) and 1.4×10^8 spleen cells collected from a mouse 4 days after the last mosquito bite. The production of sporozoite antibodies by individual hybridoma cultures was assessed by indirect immunofluorescence (*4*), glutaraldehyde-fixed sporozoites being used as antigens, and also by the CSP reaction (*3*) with viable parasites.

The supernatants of 600 cultures were screened, and five were found to produce sporozoite antibodies 2 weeks after the fusion procedure. However, only one hybrid cell line (3D11) was successfully expanded; this line has been maintained in Dulbecco's modified Eagle medium containing 10 percent fetal calf serum (*8*). The hybrid cells were also inoculated intraperitoneally into BALB/c mice, which resulted in the rapid formation of ascites containing high concentrations of sporozoite antibodies (Table 1). The CSP and immunofluorescence titers obtained with the serum and the ascitic fluid of hybridoma-inoculated mice were from 10^2 to 10^3 times higher than the titer of the serum of mice immunized repeatedly with irradiated sporozoites. The monoclonal antibodies were capable of neutralizing sporozoites in vitro, abolishing their infectivity. In four experiments, *P. berghei* sporozoites were incubated for 45 minutes at room temperature either with the 3D11 culture fluid or with

Table 1. Titer of *P. berghei* antisporozoite antibodies produced by hybridoma cells and by sporozoite-immunized mice. Viable and glutaraldehyde-fixed sporozoites were used as antigen for the CSP and immunofluorescence reactions, respectively.

Source of antibody	CSP titer	Immunofluorescence titer
Hybridoma		
Culture fluid*	1 : 16	1 : 2,048
Serum from mice with solid tumors†	1 : 400	1 : 1,160,000
Serum from mice with ascites†	1 : 1,600	1 : 800,000
Ascites‡	1 : 1,600	1 : 1,600,000
Mice immunized with *P. berghei* sporozoites	1 : 32	1 : 4,000

*Culture fluids were titrated at the time of maximum cell growth. †Serum was collected from BALB/c mice 9 to 10 days after they were inoculated subcutaneously or intraperitoneally with 7×10^6 3D11 cells. ‡Ascitic fluid was obtained from BALB/c mice 1 to 2 weeks after they were inoculated with 7×10^6 3D11 cells.

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