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Homogeneous Antibodies Directed Against Human Cell Surface Antigens: I. The Mouse Spleen Fragment Culture Response to T and B Cell Lines Derived From the Same Individual

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The use of the mouse spleen fragment culture system is extended to the production of antibodies to human lymphoblastoid cell lines. These antibodies were tested for reactivity against the immunizing cell line, and against a second cell line which had been derived from the same human blood sample. Many of the antibodies were found to discriminate between the 2 isogenic lines. These results demonstrate the potential of the mouse spleen fragment culture system to provide homogeneous reagents which detect distinguishing markers on closely related human cells.

Key words: antibody, human T cell, human B cell

Among the different techniques that have been described for the in vitro production of antibody (1–3), the mouse spleen fragment culture system (3) is of particular value because it permits one to routinely obtain the antibody product of a single clone of responding B cells. The monoclonal nature of this response (4–7) has a number of important consequences. Firstly, knowing that the responding cells are the clonal progeny of a single responding B cell allows one to infer some of the properties of that cell, such as its frequency, specificity, or requirements for triggering (8, 9). Secondly, because the antibody produced by each fragment is homogeneous with respect to its specificity, spleen fragment cultures provide a source of material for the study of the antibody molecule itself (6, 7). In both of these cases, molecules of known structure have been the antigens of choice. Finally, the homogeneous antibody may be used as a probe for the study of the antigen to which it is directed. It is only relatively recently, with the development of techniques for the production and assay of responses to complex antigens, that this final aspect of the fragment culture response is beginning to be exploited. Antigens that have been used in this context include influenza virus (10, 11), murine tumor cells (12), and, most recently, in our laboratory, human tumor cells (13) and cell lines.

Previous work in this laboratory has established the feasibility of using the mouse spleen fragment culture system to obtain monoclonal antibodies to human cell surface

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antigens; of particular significance was the finding that many of the antibodies raised could differentiate between presumably related cell types, such as between chronic lymphocytic leukemias from different patients (13). In order to further explore the ability of the mouse to recognize human cell surface antigens, we have studied the mouse spleen fragment culture response to a pair of human lymphoblastoid cell lines derived from the same individual. One of these, 8392, has been characterized as a B cell line, and the other, 8402, as a T cell line (14, 15). Thus, although these 2 lines have the same genetic background, they also differ in many of their surface characteristics. The question we have asked is: How many of the mouse monoclonal antibodies produced against one of these lines will be directed against common antigens, and how many of the antibodies will be able to discriminate between the 2 cell lines?

MATERIALS AND METHODS

Cell lines. Four human lymphoblastoid cell lines, 8402, 8392, H-SB2, and SB, were used in these experiments. 8402 and 8392 are T and B cell lines derived from the same human blood sample; H-SB2 and SB are T and B cell lines derived from a second human blood sample. The derivation, characterization, and conditions for culturing these lines have been described previously (14–16).

The fragment culture system. The use of the mouse spleen fragment culture system to obtain antibodies to human cell surface antigens has been described previously (13). Briefly, for each cell line studied, (Balb/c $\times$ C57 B1/6) F1 mice were immunized and boosted, intraperitoneally, with 10^7 human cells. From 2 to 4 weeks following the boost, 0.5-50 $\times$ 10^6 viable spleen cells from the immunized donors were transferred, intravenously, into lethally (1,300 rad) irradiated recipients. Within a few minutes the irradiated recipients also received an intravenous dose of 10^7 of the immunizing human cells. Within 24 h the recipient spleens were chopped into 1 mm^3 fragments, and the fragments cultured individually in Linbro plates in MEM-10% agamma horse serum-glutamine-gentamicin. Culture supernatants were harvested at 3–4 day intervals for the first month of culture, and stored at $-70°C$ until assay.

Goat anti-mouse kappa. A goat was immunized with the Fab fragments of a purified mouse anti-DNP antibody (17). The globulin fraction of the resulting goat anti-mouse Fab antiserum was purified by ammonium sulfate precipitation (at 40% saturation) and passed over an affinity column (18) of purified W3207 (a mouse $\kappa\alpha$ myeloma) protein, which was the generous gift of Ms. Carol Nottenburg. The bound goat anti-mouse κ was eluted from this column with 0.1 N acetic acid, and was radioiodinated as described previously (19) for use in the radioimmunoassay (see below).

Radioimmunoassay. The radioimmunoassay for the detection of antibody to human cell surface antigens has been described previously (13). Briefly, 25 μl of supernatant fluid was incubated with 25 $\times$ 10^4 glutaraldehyde-fixed target cells for 2 hr at room temperature; the cells were washed free of unbound material, and then incubated with ^{125}I-goat anti-mouse κ overnight at $4°C$. The cells were then washed extensively, and the amount of label bound to the pellets was counted in a gamma counter.

Data analysis. In each experiment, 50–100 control fragments were cultured. These were fragments from the spleens of irradiated animals which had been given the stimulating dose of antigen, but no donor spleen cells. At least 12 control fluids were included in each assay, and the mean and standard deviation of the control binding activity was computed. In assessing the binding activity of experimental supernatant fluids, those whose binding

TABLE I. Antigen Dependence of the Fragment Culture Response to Human Cell Lines

Stimulator cells	Positive fragments
	Experiment 1
	Stimulator cells = 8402[a]
0	0/42 = 0%
10^5	2/96 = 2%
10^6	5/96 = 5%
10^7	3/84 = 4%
	Experiment 2
	Stimulator = 8392[b]
0	0/45 = 0%
10^5	3/90 = 3%

[a]Donor mice were primed and boosted with 10^7 8402 cells; 2×10^6 donor spleen cells were transferred to irradiated recipients, and $0-10^7$ 8402 cells were injected within a few minutes. One day later, the recipient spleens were chopped and cultured as described in Materials and Methods.
[b]Same as 1, except 8392 cells were used to immunize, boost, and stimulate.

TABLE II. Donor Cell Dependence of the Fragment Culture Response to Human Cell Lines*

	Positive fragments			
	Stimulator = 8402[a]		Stimulator = 8392[a]	
Donor cells	Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	0/48 = 0%	–[b]	0/45 = 0%	–
0.5×10^6	1/96 = 1%	–	0/96 = 0%	–
2×10^6	3/84 = 4%	–	1/96 = 1%	–
$10-12 \times 10^6$	6/143 = 4%	–	3/96 = 3%	–
30×10^6	–	29/83 = 35%	–	5/96 = 5%
50×10^6	–	–	12/41 = 29%	–

*From $0-50 \times 10^6$ donor spleen cells were transferred to irradiated recipients, and 10^7 stimulator cells were injected within a few minutes. One day later, the recipient spleens were chopped and cultured as described in Materials and Methods.
[a]Different groups of donor mice were used for Experiments 1 and 2.
[b]Not done.

activity fell within 2 standard deviations of the control mean were scored as negative, and those whose binding activity fell at least 5 standard deviations from the control mean were scored as positive. Binding activities falling between these 2 cut-off points were not scored.

RESULTS

The Mouse Spleen Fragment Culture Response to the Human Lymphoblastoid Cell Lines 8402 and 8392

In order to determine the appropriate conditions of culture, mouse spleen fragment culture responses to either 8402 or 8392 were established using varying doses of donor

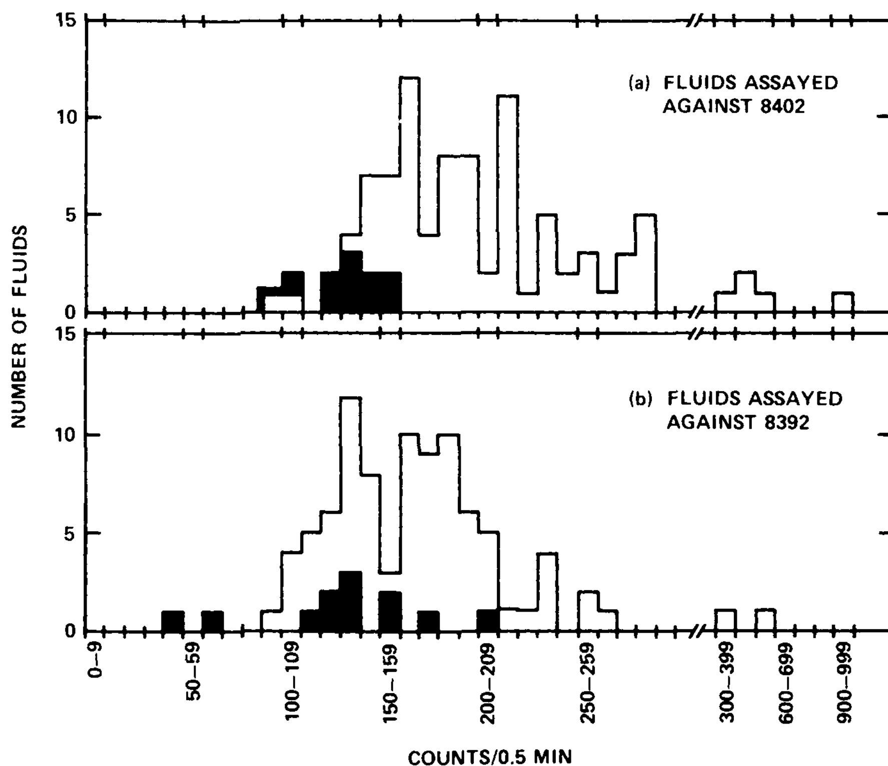

Fig. 1. Distributions of binding activities against 8402 (the immunizing cell) and its isogenic partner, 8392. Donor mice were immunized and boosted with 8402. Recipient mice received $0.5-10 \times 10^6$ donor spleen cells and a stimulating dose of 8402 cells within a few minutes. Recipient spleens were chopped and cultured as described in Materials and Methods. Day 7 supernatant fluids were assayed against a) 8402 or b) 8392.

and stimulating cells. The supernatant fluids were collected on day 7 after the beginning of culture and assayed in the radioimmunoassay for antibody activity against the immunizing cell line. Table I shows the number of responding fragments obtained when 2×10^6 donor cells and from $0-10^7$ stimulator cells were given. Table I verified that the response is antigen-dependent and that 10^7 cells is a great excess of stimulating cells.

Table II shows the number of responding fragments obtained when the stimulating dose was held constant and the dose of donor cells varied over the range $0.5-50 \times 10^6$ cells. Table II confirms that the response is also donor cell-dependent. Moreover, Table II shows that at doses of up to $10-12 \times 10^6$ donor spleen cells when 8402 is the immunogen, and up to 30×10^6 donor spleen cells when 8392 is the immunogen, the cloning efficiency is low enough so that the probability of one fragment containing 2 responding B cells is low (10).

The Broad Specificity of the Response to 8402

In order to assess the broad specificity of culture fluids obtained against 8402, 90 such fluids were assayed against both 8402 and its isogenic partner, 8392. Each fluid was assayed at 3 dilutions: undiluted, 1:4, and 1:16. The results obtained with the un-

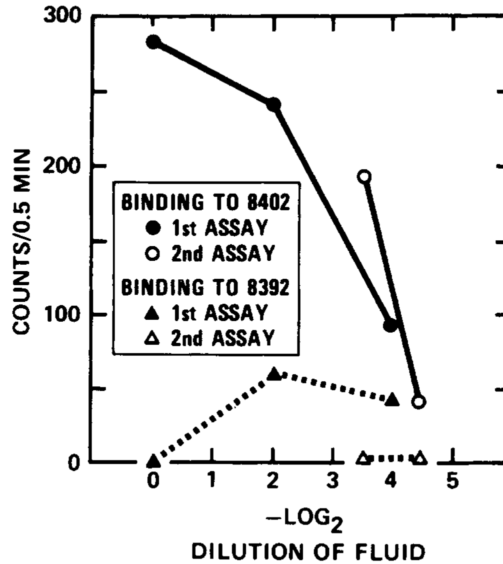

Fig. 2. Example of a discriminator culture fluid. This clone was raised against the human T cell line 8402. The supernatant fluid was positive against the immunizing cell and negative against the isogenic B cell, 8392 (see text). Circles show binding to 8402, triangles show binding to 8392; filled symbols represent first assay, open symbols represent second assay. In each case, the mean binding of 12 control fluids was subtracted from the total counts. Means and standard deviations of the control values were: first assay: 129 ± 20 (8402), 129 ± 43 (8392); second assay: 81 ± 30 (8402), 78 ± 13 (8392).

diluted fluids are shown on Fig. 1. The backgrounds seen when 12 control fluids (see Materials and Methods) were assayed against each of the 2 cell lines are also shown in Fig. 1 (shaded areas). It is apparent from Fig. 1 that, while the majority of the fluids tested bound to the immunizing T cell line (8402), when the same fluids were assayed against 8392 relatively few showed levels of binding to the target cells above background levels. A similar analysis of the data obtained with the diluted fluids gave the same results (data not shown).

In order to analyze this data in another way, individual culture fluids were classed as *discriminators* if they were positive against 8402 at at least 2 of the dilutions tested, and negative against 8392 at all 3 dilutions. (Responses were scored as positive or negative as described in Materials and Methods.) Culture fluids were classed as *nondiscriminators* if they were positive at at least 2 dilutions against both cell lines. Of the 90 culture fluids that were studied, 11 fell into either of these categories; of these 11 culture fluids, 9 were discriminators and 2 were nondiscriminators. (None of the 90 culture fluids was negative against 8402 but positive against 8392.) An example of a discriminator culture fluid is shown in Fig. 2.

The Response to 8392.

In order to determine the specificity of culture fluids obtained when a B cell was the immunizing cell, 5 culture fluids which had been raised against 8392 and originally scored as positive against that cell were reassayed against both 8392 and its isogenic partner, 8402. Using the criteria described in the preceding paragraph, all 5 of these fluids were found to be discriminators; an example of one of these is shown in Fig. 3.

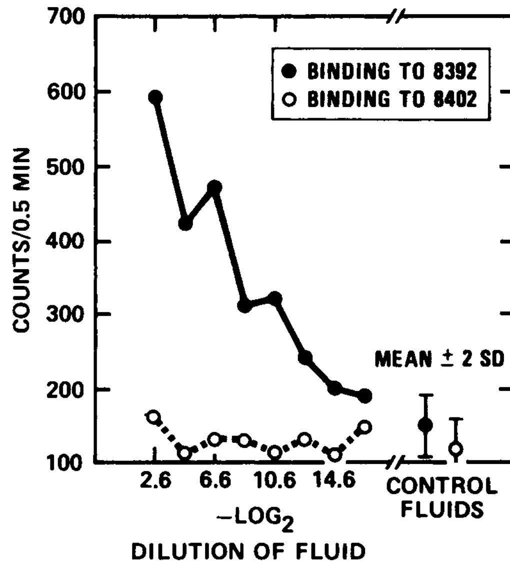

Fig. 3. Example of a discriminator culture fluid. This clone was raised against the human B cell line 8392. The supernatant fluid was positive against the immunizing cell and negative against the isogenic T cell, 8402. Filled circles show binding to 8392, open circles show binding to 8402. The mean and 2 standard deviations of the binding of 12 control fluids are also shown.

Other Responses

The results presented thus far have been taken from experiments in which the cloning efficiency was low. Even where the cloning efficiencies were much higher, so that one would expect the majority of the fragments to contain more than one responding B cell (10), there were many fragments whose fluids showed more binding activity for the immunizing cell than for its isogenic partner. In one group of experiments, 96 fluids were raised against 8402, and 77 of these (83%) were found to be positive against the immunizing cell. Of these 77 fluids, 7 showed quantitatively more binding activity against 8402 than against its isogenic partner, 8392. The binding activities of 4 of these culture fluids are illustrated in Fig. 4. (None of the 77 fragments analyzed showed more binding activity against 8392 than against 8402.)

A final group of experiments involved a second isogenic T-B pair, H-SB2 and SB. In this case, the B cell (SB) was used as the immunogen, and of 94 culture fluids that were screened for antibody activity, 92 (98%) were found to be positive against the immunizing cell. Of the 92 positive fluids, 62 showed quantitatively more activity against the immunizing B cell (SB) than against its isogenic partner (H-SB2). The binding activity of 4 of these fluids is shown in Fig. 5. (None of the fluids showed more binding activity against H-SB2 than against SB.)

DISCUSSION

Having previously shown that the mouse can make antibodies which discriminate between leukemias from different individuals (13), we wished to extend our analysis of the mouse B cell repertoire against human cells by studying the mouse spleen fragment culture

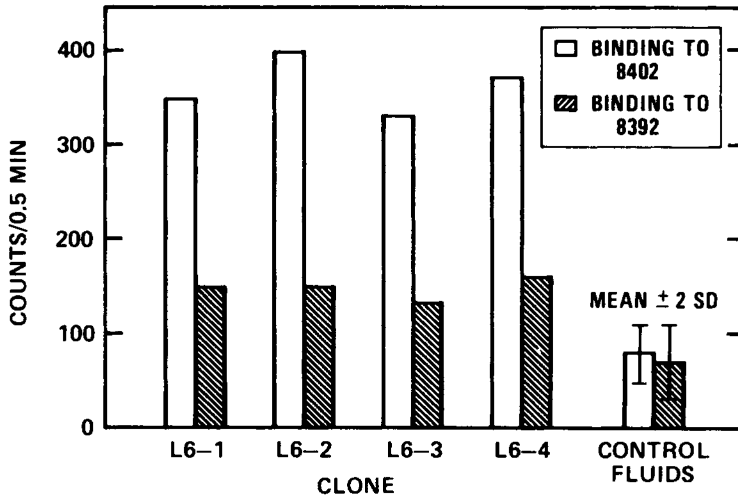

Fig. 4. Examples of culture fluids which show quantitative differences in binding to isogenic cell lines. Each clone was generated against the human T cell line 8402. The culture fluids were all positive against the immunizing cell and either negative or not scoreable against the isogenic partner, 8392. (For explanation of scoring of fluids see Materials and Methods.) In each pair, open bar shows binding to 8402, shaded bar shows binding to 8392. Mean and 2 standard deviations of the binding of 12 control fluids are also shown.

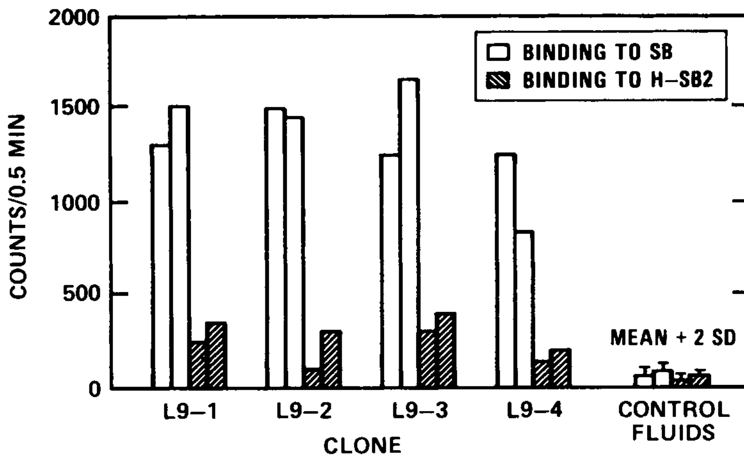

Fig. 5. Examples of culture fluids which show quantitative differences in binding to isogenic cell lines. Each clone was generated against the human B cell line SB. The culture fluids were positive against both the immunizing cell and the isogenic partner, but showed large quantitative differences in the amount of binding to the 2 cell lines. In each group of bars, open bars show binding to SB, shaded bars show binding to H-SB2. In each group, first and third bars show data from first assay, second and fourth bars show data from second assay. The mean and 2 standard deviations of the binding of 35–37 control fluids are also shown.

response to pairs of human cells with the same genetic background. In this paper, we have described conditions under which antibodies can be raised against human lymphoblastoid cell lines in the mouse spleen fragment culture system, and have presented our preliminary results showing the specificity of the responses obtained. We have shown that, at low

cloning efficiency, many of the clones produce antibody which discriminates between the immunizing cell and its isogenic partner, and that, even at high cloning efficiency, many clones are found which show quantitative differences in their binding to the 2 cell lines.

We wish to stress the reciprocity of the responses that we have described. When the immunizing cell line was a human T cell line, many of the resulting culture fluids were positive for that cell line and negative for an isogenic B cell line, and none of the fluids reacted only with the B cell line. Conversely, when the immunizing cell line was a B cell line, we found many fluids which reacted only with the B cell line, and none which reacted only with an isogenic T cell line. These findings are in agreement with other reports showing that isogenic human T and B cell lines each bear distinguishing antigenic markers (15, 16, 20). The importance of the present work is that we have shown the feasibility of using the mouse spleen fragment culture system to obtain homogeneous antibodies to these markers. These antibodies may now serve as reagents for the characterization of the surface of human T and B cells, and for the identification of subpopulations of normal and transformed T and B cells.

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