Serotherapy of L1210 murine leukaemia—reasons for ineffectiveness of in vivo treatment by L.1 monoclonal antibody

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Summary A monoclonal antibody (L.1), reacting in vitro specifically with L1210 leukaemia cells in a complement-dependent cytotoxicity assay (CDC), has been exploited for serotherapy studies. Different regimens of L.1 treatment of CD2F1, mice bearing the semi-syngeneic L1210 leukaemia did not prolong the life span of tumor-bearing animals. Moreover, the administration of L.1 did not enhance the antitumour effects of cyclophosphamide. Studies of in vivo localization showed that L.1 was able to bind specifically to L1210 leukaemic cells, although 30-40% of the cells remained negative. The presence of L.1 in mouse blood was demonstrated up to 15 days after the inoculation. On the other hand, in vivo administration of L.1 was probably accompanied by loss of the cytotoxic activity, perhaps through a mechanism of complement inactivation, since the presence of undiluted normal mouse serum in a CDC assay inhibited the cytotoxic activity of L.1. Moreover, serum from L.1-treated mice did not display any cytotoxic activity, although the presence of the antibody could be demonstrated by indirect immunofluorescence.

Shedding of the antigen defined by L.1 was probably not responsible for the failure of the serotherapy, since the L.1 neutralizing antigen could be found in body fluids only long after the start of therapy.

The treatment of malignant diseases by passive administration of antibodies (Wright & Bernstein 1980) has been pursued as a promising therapy, although the life span after antiserum administration has not so far been improved. The failure of conventional serotherapy has been mainly attributed to the low specificity of the antisera and the poor immunogenicity of tumour-associated antigens (TAA). The new era in serology since hybridoma technology (Köhler & Milstein 1975) was devised might be exploited for new modalities of cancer immuno therapy.

Tumour-specific antigens and specific antibodies are the basic necessities for successful passive immunotherapy of cancer. Although tumour-specific antigens in human tumours (Metzgar et al., 1981; Iwaki et al., 1982; Ball et al., 1982) have not yet been definitely demonstrated, monoclonal antibodies (MAb) highly specific to TAA (Gunn et al., 1981) or to tissue and differentiation antigens (Kirch & Hammerling, 1981) might provide optimal tools to study the anti-tumour efficacy of passive serotherapy (Kirch & Hammerling, 1981; Bernstein et al., 1980; 1980; Scheinberg & Strand 1982; Ritz et al., 1981; Trowbridge & Lopez, 1982; Sears et al., 1982).

In a previous report from this laboratory (Testorelli et al., 1982), the production and the characterization of a MAb reacting specifically with the cells of the L1210 murine leukaemia (L.1) was described. The L.1 MAb displayed in vitro very high and specific complement-dependent cytotoxicity against L1210 cells. On the other hand, it did not react with normal adult and foetal murine tissues, with cells from several independent haplotypes or with a number of chemical- and virus-induced experimental tumours.

In the present report, the therapeutic effects of the administration of L.1 alone or combined with chemotherapeutic agents on the survival rates of leukaemic mice are presented. Possible reasons for the ineffectiveness of serotherapy with L.1 were also investigated and are discussed.

Materials and methods

Animals and tumours

Inbred DBA/2, Balb/c and hybrid (Balb/c x DBA/2) CD2F1, male mice, 10–15 weeks old, were obtained from Charles River (Calco, Italy). L1210/Cr leukaemia was maintained by weekly passages (i.p.) into compatible CD2F1 mice. Balb/c or CD2F1
mice were used to produce antibody-containing ascites.

**Hybrid cell lines and production of ascitic fluid**

The production of the hybridoma cell line secreting the monoclonal antibody L.1 (IgM) to a surface antigen of L1210 cells has been described previously (Testorelli et al., 1982). The hybridoma was maintained in culture in RPMI 1640 medium (Eurobio, Paris), supplemented with 10% heat-inactivated foetal calf serum (FCS, Seromed GmBh, München, Germany) and 100 μl ml⁻¹ gentamycin (Seromed). The supernatant from the cultures was stored at −70°C. For production of ascitic fluid, Balb/c or CD2F1 mice, preprimed with pristane (0.5 ml, i.p.), were injected i.p. with 10⁶ hybrid cells. Ascitic fluid was collected after 15–20 days, centrifuged to remove the cells and frozen at −70°C until use. The cytotoxic titers of both supernatant and ascitic fluid were assessed by a complement-dependent cytotoxicity assay (see below) using NaCr-labelled L1210 cells as target. Titers are expressed as the highest dilution that killed 50% of leukemic cells. When not otherwise specified, the cytotoxic titer was 1:64 for the supernatant from the hybridoma culture and 10⁻⁶ for ascites.

**Complement-dependent cytotoxicity assay (CDC)**

NaCr-release assay was performed as described elsewhere (Testorelli et al., 1982). Briefly, 25 μl of target cells labelled with NaCr sodium chromate were incubated with 25 μl of the L.1 (P3X63/Ag8 supernatant was used as control) for 45 min at 37°C. Rabbit complement diluted 1:5 was then added (25 μl) and the cells were incubated for 30 min at 37°C in a humidified 5% CO₂ atmosphere. The percentage of NaCr release into the supernatants of triplicate samples was calculated according to Ozato et al. (Ozato et al., 1980).

**Indirect membrane immunofluorescence (IMF)**

The L1210 cells were tested after extensive washing for their viability (cells <95% viable were excluded) and incubated with 20 μl of supernatant from the hybridoma culture for 40 min at 4°C. The cells were washed twice and subsequently incubated with 0.15 of a 1:10 dilution of fluorescein isothiocyanate-conjugated (FITC) rabbit anti-mouse IgG (Cappel Lab., Cochravanille, PA) for 30 min at 4°C. The cells were washed 3x and analyzed under a microscope with epifluorescence optics and in the fluorescence-activated cell sorter (FACS II, Becton Dickinson, Mountain View, Calif.).

**Serotherapy of L1210-bearing mice with L.1**

CD2F1 were challenged i.p. with different numbers of L1210 cells (day 0). Experimental animals were given injections of L.1 (ascitic fluid containing 30mg MAb ml⁻¹) alone or combined with rabbit complement, starting 24h after challenge. Different schedules of treatment were adopted. In the combined therapy, L.1 was given i.p. with cyclophosphamide 120mg kg⁻¹ s.c. Tumour growth was evaluated by recording the median survival time (MST) and the number of dead animals per group (D/T) of control and treated animals.

**In vitro evaluation of serotherapy**

**In vivo binding of L.1 to L1210** At different intervals after the i.v. infusion of L.1 into L1210-bearing mice, tumour cells were collected from peritoneal cavity and divided into 2 pools. The first pool was analysed by IMF for the presence of L.1 on the cells surface. The cells from the second pool were labelled with NaCr and assayed for their susceptibility to lysis by the CDC system.

Detection of L.1 in the serum of normal mice Blood samples from CD2F, mice infused i.v. with L.1 were collected at different times after the injection and the serum tested for the presence of L.1 by both the IMF and CDC systems.

Detection of circulating antigen in L1210 mouse fluids The presence of the antigen defined by L.1 in the serum and in the ascites of L1210-bearing mice was detected by incubating at 4°C for 45 min an appropriate dilution of L.1 (supernatant from hydridoma culture) with an equal volume of serum or ascites (undiluted, 1:2, 1:4 etc.) from mice at different days after tumour challenge. The resulting L.1 cytotoxic activity was assayed against L1210 target cells.

Inhibition of complement activity by normal mouse serum Normal sera from different strains of mice were mixed for 45 min at 4°C with NaCr-labelled L1210 cells (10⁶) and L.1 culture supernatant. The mixture (50 μl) was seeded in a 96-well microplate, complement was added (25 μl) and the cytotoxic activity of L.1 was evaluated as reported above (CDC).

**Results**

**Effects of L.1 administration on survival of leukaemic mice**

The results of passive serotherapy of leukaemic mice with L.1 and rabbit serum, as source of
Table I Serotherapy of L1210-leukaemic CD2F₁ mice with L.1 and complement

| L1210 Challenge, i.p. | L.1* Day +1 ml/mouse, i.p. | Rabbit complementb Day +1 ml/mouse, i.p. | MSTc (range) | D/Td |
|----------------------|--------------------------|----------------------------------------|-------------|------|
| 10⁴*                 | —                        | —                                     | 12(11-14)   | 8/8  |
| 10³*                 | 1.0                      | 0.4                                   | 13(11-14)   | 8/8  |
| 10⁴*                 | 0.01                     | 0.4                                   | 13(12-14)   | 8/8  |
| 10³*                 | 0.1                      | 0.4                                   | 12(12-13)   | 8/8  |
| 10³*                 | 1.0                      | 0.4                                   | 12(12-14)   | 8/8  |

*The source of L.1 was an ascitic fluid containing 30 mg antibody ml⁻¹ with a cytotoxic titre of 10⁻⁶.

bComplement activity was checked in vitro.

cMST: Median Survival Time (days).

dD/T: Dead Mice/Treated Mice.

*The same treatment to mice challenged with 10³ L1210 cells was ineffective.

whether the MAb acted synergistically with a chemotherapeutic agent, leukaemic mice were treated with an effective dose of cyclophosphamide plus L.1. As shown in Table III serotherapy did not significantly increase the survival time over cyclophosphamide alone.

L.1 binding to L1210 cells in vivo

In order to study the reasons for the failure of L.1 therapy, experiments were designed to determine whether the L.1 could bind specifically to tumour cells in vivo. Figure 1 shows that, although 24 h after L.1 administration the majority of L1210 cells were stained, a remarkable proportion of unstained cells was still detectable. Moreover, the fluorescence intensity of stained cells was never >20% of that of L1210 cells treated in vitro with L.1. The % of +ve cells and fluorescence intensity were completely restored when L1210 cells interacting with L.1 in vivo were further exposed to the MAb in vitro.

Results of time-course experiments are also illustrated in Figure 1. The % of L.1 +ve cells analysed by automatic flow fluorocytometry peaked

Table II Serotherapy of L1210-leukaemic CD2F₁ mice with L.1 and complement

| L.1* Days and route of treatment 0.5 ml/mouse | MST (Range) | D/T |
|---------------------------------------------|-------------|-----|
| L1210 Challenge i.p.                        |             |     |
| 10⁴*                                        | —           | 13(12-13) | 8/8 |
| 10³*                                        | 1, 3.5 i.p. | 14(11-15) | 8/8 |
| 10³*                                        | 1, 3 i.v.   | 12(11-13) | 8/8 |
| 10³*                                        | 1-7 i.p.    | 14(13-15) | 8/8 |
| 10³*                                        | 1-7 i.v.    | 14(12-15) | 8/8 |

*See Table I. Rabbit serum as source of complement (0.4 ml/mouse, i.p.) was given after every L.1 inoculation.

*The same treatment in mice challenged with 10³ L1210 cells was ineffective.

Table III Chemotherapy plus serotherapy of L1210-leukaemic CD2F₁ mice

| L1210 Challenge i.p. | Cy₆ Day 0 120 mg Kg⁻¹ s.c. | L.1* Day +1 0.5 ml/mouse i.v. | MST (Range) | D/T |
|----------------------|----------------------------|--------------------------------|-------------|-----|
| 10⁵                  | —                          | —                              | 9(8-10)     | 8/8 |
| 10⁴                  | +                          | —                              | 12(11-14)   | 8/8 |
| 10³                  | −                          | +                              | 10(9-10)    | 8/8 |
| 10³                  | +                          | +                              | 11(10-13)   | 8/8 |

*See Table I. Rabbit serum as source of complement (0.4 ml/mouse, i.p.) was given after every L.1 inoculation.

*The same treatment in mice challenged with 10³ L1210 cells was ineffective.

*Cy: Cyclophosphamide.

*L.1 and rabbit complement; see footnote Table II.
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Figure 1 Binding of L.1 to L1210 cells in vivo. L1210 leukaemic cells, obtained from tumour-bearing animals at different intervals from an i.v. administration of L.1 (0.2 ml of ascites diluted 1:4) were analyzed for the presence of the MAb on their surface by IMF. The fluorescence staining (---) and the fluorescence intensity (----) of L1210 leukaemic cells treated in vivo with L.1 (○) and further exposed to L.1 in vitro (○) are reported. Tumour cells from untreated animals were used as −ve controls.

24 h after the L.1 inoculation and decreased gradually, although 15% of +ve cells were still detected 6 days after the treatment. As shown in Table IV, the amount of L.1 bound to L1210 cells after in vivo treatment was dependent upon the dose of the antibody injected. Findings in keeping with those reported in Figure 1 were obtained in the cytoxic assay. (Figure 2). A % of tumour cells derived from L1210-bearing mice previously treated with L.1 underwent lysis upon in vitro incubation with rabbit complement. However, full susceptibility to complement-mediated lysis was obtained upon cell re-exposure to L.1 in vitro.

Studies of L.1 properties after in vivo administration

The time course of blood L.1 levels in CD2F₁ mice, as evaluated by IMF (dashed line) or by the ⁵¹Cr-release assay (full line), was studied (Figure 3).

Table IV Binding of L.1 to L1210 cells in vivo; dose-response relationship

| L.1<sup>§</sup> antibody dilution | Fluorescence % stained cells<sup>a</sup> | Dye Test %dead cells<sup>a</sup> |
|----------------------------------|--------------------------------------|----------------------------------|
| −                                | 3 ± 0.1                              | 9 ± 0.3                          |
| 10<sup>9</sup>                   | 64 ± 6.9                             | 78 ± 0.8                         |
| 10<sup>7</sup>                   | 67 ± 6.2                             | 75 ± 2.5                         |
| 10<sup>5</sup>                   | 34 ± 7.6                             | 33 ± 1.3                         |
| 10<sup>3</sup>                   | 18 ± 4.1                             | 24 ± 0.9                         |
| 10<sup>4</sup>                   | 6 ± 0.3                              | 19 ± 0.1                         |

<sup>a</sup>Mean ± s.e.

<sup>§</sup>Tumour-bearing mice were infused i.v. with L.1 (ascites, 0.5 ml/mouse) and, 24 h later, L1210 leukaemic cells were collected and assayed for the presence of L.1 by IMF and by the Dye exclusion test of Gorer & O’Gorman (1956).

Figure 2 In vitro lysis of L1210 cells treated in vivo with L.1. The in vivo treatment was performed as reported in Figure 1. L1210 tumour cells collected from L.1 treated mice were labelled with ⁵¹Cr and assayed in the CDC assay in the presence of rabbit complement alone (○) and after further exposure to L.1 in vitro (○) (supernatant from culture). Controls were performed using L1210 cells from untreated mice incubated in vitro with rabbit complement alone (×) or with L.1 + complement (+).

Figure 3 Blood kinetics of L.1 in CD2F₁ mice. Serum samples from CD2F₁ mice i.v. infused with 1 ml of L.1 (0.2 ml of ascites diluted 1:4) were analyzed for the presence of the MAb in the IMF (○---○) and CDC (○---○) assays at different times after inoculation.
L1210 cells incubated with serum from L.1 treated mice were stained positively in the IMF assay. After 4 days, the binding activity dropped progressively, although it was still detectable 15 days after L.1 inoculation. In sharp contrast, no cytotoxic activity was present in serum from L.1-treated mice. The possibility that complement inactivation by mouse serum was responsible for the in vivo loss of L.1 cytotoxic activity, was investigated. Serum samples from CD2F1 mice inoculated i.v. with rabbit complement did not show any complement activity in the CDC (data not shown). Moreover, the presence of undiluted mouse serum in the CDC assay inhibited the cytotoxic reaction, whatever the source of complement (Table V). However, a normal lytic reaction was observed when the mouse serum was removed before the addition of complement.

L1210 antigen in mouse fluids

The detectability of antigen recognized by L.1 shed by tumour cells into the blood or the ascites of

Table V Inhibition of complement activity by normal mouse serum

| Serum from mouse strain | Source of complement* | cpm ± s.e. | % 51Cr release |
|-------------------------|-----------------------|------------|----------------|
| —                       | rabbit                | 2495 ± 207 | 80.3           |
| CD2F1                   | rabbit                | 430 ± 38   | 0.2            |
| CD2F1                   | rabbit                | 426 ± 41   | 0              |
| CD2F1                   | rabbit                | 448 ± 39   | 0              |
| CD2F1                   | guinea pig            | 428 ± 46   | 0              |
| CD2F1                   | guinea pig            | 431 ± 37   | 0.2            |
| CD2F1                   | human                 | 445 ± 48   | 0.8            |
| DBA/2                   | rabbit                | 412 ± 40   | 0              |
| Balb/c                  | rabbit                | 429 ± 45   | 0              |
| C57Bl/6                 | rabbit                | 410 ± 28   | 0              |
| C3H/f                   | rabbit                | 425 ± 33   | 0              |
| CD2F1(w)                | rabbit                | 2360 ± 250 | 75.5           |
| CD2F1(w)                | guinea pig            | 2405 ± 198 | 77.2           |
| CD2F1(w)                | human                 | 2381 ± 279 | 76.3           |

The cytotoxic activity of L.1 was evaluated in a CDC with L1210 target cells, in the presence of normal serum from different strains of mice as described in Materials and Methods. When indicated (w), L.1 and mouse serum were washed off before adding the complement. L.1 (supernatant from hybridoma culture) was present in all samples. Replacement of L.1 with rabbit anti mouse serum caused similar results.

*Sources of rabbit complement were selected from non-cytotoxic blood samples from our breeding colony. Guinea pig sources were purchased (Bio Mérieux, France). Human complement was from healthy donors. All samples were assayed in vitro for complement activity before use, and for each complement the appropriate dilution was selected. This Table uses rabbit complement as a +ve control.

L1210-bearing mice was examined. Inhibition of cytotoxic activity of L.1 by serum and ascites fluid from leukaemic mice obtained at varying intervals after the challenge is reported in Figure 4. The inhibitory capacity of mouse fluids, not detectable until 4 days after the tumour inoculation, increased rapidly thereafter and was more impressive for the ascites fluid than for the serum. Titration of soluble antigen (Figure 5) showed that a relatively large amount of free antigen was detectable in the ascites fluid on day 7, whereas a lesser degree of inhibitory activity was demonstrable on day 5.

Figure 4 Inhibition of the cytotoxic activity of L.1 by ascites and serum from L1210-bearing mice. The supernatant from the hybridoma culture was incubated at 4°C for 45 min with the serum (●—●) and the ascites (○—○) from L1210-bearing mice on different days after the challenge i.p. with 10⁴ leukaemic cells. The mixture was added to L1210-labelled cells and incubated at 37°C (45 min). After washing an appropriate dilution of complement was added as described for the CDC assay. The results are expressed as % inhibition of L.1 cytotoxicity.

Discussion

In principle, specific and cytotoxic MAb should provide selective bullets for the in vivo eradication of cancerous cells leaving normal cells undamaged. These theoretical conditions, hypothesized since the Ehrlich era, were obtained in our laboratory by the production of L.1. In fact, L.1 is absolutely specific to L1210 leukaemic cells and as an IgM immunoglobulin displays great efficiency in fixing complement (Testorelli et al., 1982). Furthermore, since the CD2F1 mice used in these studies were of
identical haplotype to that of the L.1 hybridoma cells, strictly syngeneic conditions could be obtained. However, even under these optimal theoretical conditions, L.1 serotherapy was almost completely ineffective in increasing the survival time of L1210-bearing CD2F1 mice. Variations of the schedule of route or treatment, of the amounts of antibody or of complement were consistently unsuccessful. One reasonable cause for failure, the low level of endogenous complement in mice, was counteracted by excessive inoculation of exogenous complement, but this manoeuvre was without improvement. Since the activation of an endogenous immune response (Kirch & Hammerling, 1981) is a possible effector mechanism of serotherapy, L.1 might synergize with an effective antitumour drug. Following this line, a number of treatments with cyclophosphamide or with other anticancer compounds (not reported here) were combined with L.1 serotherapy, without any additional increase in the life-span of the mouse.

To account for the failure of L.1 serotherapy several experiments were devised. The first set was conceived to see whether or not L.1 given i.v. to tumour-bearing mice bound to peritoneal L1210 cells. Although the results were positive (Figure 1) a lower percentage than expected of dead cells and of fluorescent cells was regularly observed. It is noteworthy that the fluorescence intensity of in vivo L.1-treated cells was 80% below the intensity achieved in the in vitro-treated control cells. Decreased susceptibility to in vitro complement-mediated lysis by L1210 cells exposed in vivo to L.1 was confirmed by the 51Cr-release assay. Finally, we found that the susceptibility to both complement-mediated lysis and to staining with a second antibody were completely restored after in vitro re-exposure to L.1.

The in vivo studies suggest the following conclusions: (a) a fraction of L1210 cells did not bind L.1 in vivo; (b) the amount of L.1 bound to the surface cells +ve by IMF-was less than for in vitro-treated controls, as demonstrated by the lower fluorescence intensity; (c) the in vivo L.1 +ve cells, in spite of their low fluorescence intensity, were still susceptible to in vitro lysis in presence of an appropriate source of complement; (d) the antigenic specificity defined by L.1 did not undergo modulation, since fluorescence intensity and susceptibility to lysis were fully recovered after in vitro re-exposure to L.1: (e) cells -ve by IMF and those resistant to complement-mediated lysis might belong to the same subpopulation.

Although blood clearance of L.1 in the mouse had the half life already described by others for the same antibody class (Bernstein et al., 1980; Kirch & Hammerling 1981), blood serum samples containing L.1 and complement did not lyse L1210 cells in vitro. Furthermore, complement from different sources mixed with mouse serum in vitro was completely inactivated.

Antigen shedding cannot be responsible for the failure of L.1 serotherapy since, at the start of serotherapy, soluble antigen was not found in body fluids. L.1 inhibition by mouse serum and even more by ascites fluid from tumour-bearing mice became evident 4 days after L1210 challenge. Therefore, circulating antigen might negatively influence late treatments only.

The experimental system used in these studies although adhering to optimal theoretical conditions, did not bring about increased survival time for leukaemic mice. Incomplete antibody binding to target cells and blood inactivation of complement might be major factors in these disappointing results.

A number of claims (Kirch & Hammerling, 1981; Bernstein et al., 1980; Scheinberg & Strand 1982; Miller & Levy, 1981; Ritz et al., 1980; Trowbridge & Lopez 1982; Herlyn & Koprowski, 1981), of therapeutic benefit from the use of MAb in cancer therapy have been reported recently in the literature. Partial successes in tumour systems other than that used here could be attributed to different Ig isotypes, acting through an antibody-dependent
cellular cytotoxicity (ADCC) rather than complement-mediated lysis. The limited achievements of this approach might result in greater efforts (Pimm et al., 1982; Raso et al., 1982) to exploit monoclonal technology to carry toxic compounds specifically to their target cells.

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References

BALL, E.D., KADUSHIN, J.M., SCHACTER, B. & FANGER, M.W. (1982). Studies on the ability of monoclonal antibodies to selectively mediate complement-dependent cytotoxicity of human myelogenous leukemia blast cell. J. Immunol., 128, 1476.

BERNSTEIN, I.D., TAM, M.R. & NOWINSKI, R.C. (1980). Mouse leukemia: therapy with monoclonal antibodies against a thymus differentiation antigen. Science, 207, 68.

BERNSTEIN, I.D., NOWINSKI, R.C., TAM, M.R., MCMASTER, B., HOUSTON, L.L. & CLARK, E.A. (1980). Monoclonal antibody therapy of mouse leukemia. In: Monoclonal antibodies. Hybridomas: A New Dimension in Biological Analysis. (Eds. Kennet et al.) New York: Plenum Press, p. 275.

GORER, P.A. & O’GORMAN, (1956). The cytotoxic activity of isoantibodies in mice. Transplant. Bull., 3, 142.

GUNN, B., EMBLETON, M.J. & BALDWIN, R.W. (1981). Monoclonal antibody against a naturally occurring rat mammary carcinoma. Int. J. Cancer, 26, 325.

HERLYN, D.M. & KOPROWSKI, H. (1981). Monoclonal anticoncol carcinoma antibodies in complement-dependent cytotoxicity. Int. J. Cancer, 27, 769.

IWAKI, Y., KASAI, M., TERASAKI, P.I. & 7 others. (1982). Monoclonal antibody against A1 Lewis d antigen produced by the hybridoma immunized with a pulmonary carcinoma. Cancer Res., 42, 409.

KIRCH, M. & HAMMERLING, U. (1981). Immunotherapy of murine leukemias by monoclonal antibody. Effect of passively administered antibody on growth of transplanted tumor cells. J. Immunol., 127, 805.

KÖHLER, G. & MILSTEIN, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256, 495.

METZGAR, R.S., BOROWITZ, M.J., JONES N.H. & DOWELL, B.L. (1981). Distribution of common acute lymphoblastic leukemia antigen in non hematopoietic tissues. J. Exp. Med., 154, 1249.

MILLER, R.A. & LEVY, R. (1981). Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. Lancet, ii, 226

OZATO, K., MAYER, N. & SACHS, D.H. (1980). Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J. Immunol., 124, 533.

PIMM, M.V., JONES, J.A., PRICE, M.R., MIDDLE, J.G., EMBLETON, M.J. & BALDWIN, R.W. (1982). Tumour localization of monoclonal antibody against a rat mammary carcinoma and suppression of tumour growth with Adriamycin-antibody conjugates. Cancer Immunol. Immunother., 12, 125.

RASO, V., RITZ, J., BASALA, M. & SCHLOSSMAN S.F. (1982). Monoclonal antibody-Ricin A chain conjugate selectively cytotoxic for cells bearing the common acute lymphoblastic leukemia antigen. Cancer Res., 42, 457.

RITZ, J., PESANDO, J.M., NOTIS-MCONNARTY, J., LAZARUS, H. & SCHLOSSMAN, S.F. (1980). A monoclonal antibody to human acute lymphoblastic leukemia antigen. Nature, 283, 583.

RITZ, J., PESANDO, J.M., SALLAN, S.E. & 4 others. (1981). Serotherapy of acute lymphoblastic leukemia with monoclonal antibody. Blood, 58, 141.

SCHEINBERG, D.A. & STRAND, M. (1982). Leukaemic cell targeting and therapy by monoclonal antibody in a mouse model system. Cancer Res., 42, 44.

SEARS, H.F., HERLYN, D., HERLYN, M., STEPLEWSKI, Z., GROTZINGER, P. & KOPROWSKI, H. (1982). Ex vivo perfusion of human colon with monoclonal anticolorectal cancer antibodies. Cancer, 49, 1231.

TESTORELLI, C., MORELLI, S., GOLDIN, A. & NICOLIN, A. (1982). Characterization of a monoclonal antibody to L1210 leukemia. Br. J. Cancer, 45, 395.

TROWBRIDGE, I.S. & LOPEZ, F. (1982). Monoclonal antibody to transferin receptor blocks transferrin binding and inhibits human tumor cell growth in vitro. Proc. Natl Acad. Sci., 79, 1175.

WRIGHT, P.W. & BERNSTEIN, I.D. (1980). Serotherapy of malignant disease. Prog. Exp. Tumour Res., 25, 140.