Targeting of saporin to CD25-positive normal and neoplastic lymphocytes by an anti-saporin/anti-CD25 bispecific monoclonal antibody: in vitro evaluation

P.L. Tazza\textsuperscript{ri,}\textsuperscript{1}\textsuperscript{1}, S. Zhang\textsuperscript{1,2}, Q. Chen\textsuperscript{1,2}, S. Sforzini\textsuperscript{1}, A. Bolognesi\textsuperscript{1}, F. Stirpe\textsuperscript{1}, H. Xie\textsuperscript{2}, A. Moretta\textsuperscript{1} & S. Ferrini\textsuperscript{1}

\textsuperscript{1}Istituto Nazionale per La Ricerca sul Cancro, Genova; \textsuperscript{2}Shanghai Institute of Cell Biology, Shanghai; \textsuperscript{3}Istituto di Istologia ed Embriologia, Universita' di Genova, Italy.

Summary

This study has been designed to verify the specific toxicity of saporin, a type I ribosome-inactivating protein (RIP), with the same activity as ricin A chain, targeted by a bispecific monoclonal antibody (bimAb) recognising both the CD25 antigen and the RIP.

The CD25 antigen is expressed by lymphoid populations upon activation and by leukaemias and lymphomas with an activated membrane phenotype (Hodgkin's lymphoma, anaplastic large cell lymphoma, adult T cell leukaemia). The bimAb-saporin mixture was tested on CD25 + targets at different bimAb and saporin concentrations. Saporin, in the presence of a bimAb concentration of $10^{-5}$ M, inhibited protein synthesis by CD25 + neoplastic lymphocytes (LS40 and MT2 cell lines) with IC_{50} (concentrations giving 50% of inhibition) ranging from $8 \times 10^{-12}$ M to $3 \times 10^{-10}$ M. The saporin-bimAb mixture was also effective in blocking the phytohaemagglutinin-driven proliferation of normal lymphocytes, whereas it displayed the same level of toxicity exerted by saporin alone on an irrelevant CD25-negative cell line (EBV-infected B lymphoblastoid cell line). From these results it is possible to envisage a clinical use of this bimAb as a cytotoxic agent for CD25 + leukemias and lymphomas, as well as an immunosuppressive agent for severe immune disorders such as graft-vs-host disease (GVHD) and transplanted organ rejection.

Materials and methods

Ribosome-inactivating proteins

Saporin, PAP-S, PAP-L, momordin and gelonin were prepared as described by Barbieri et al. (1987). Ricin A chain was purchased from Sigma.

Production of anti-saporin hybridoma and of enzyme-deficient hybridoma mutants

To produce anti-saporin mAbs, 6-week-old Balb/c mice were immunised by s.c. injections of 1 mg of saporin in complete Freund's adjuvant. After 2 weeks, mice were further immunised three times with the same amount of saporin in incomplete adjuvant at weekly intervals. After 10 days mice received a booster injection of 1 mg of saporin, followed by splenectomy 3 days later. Immune splenocytes were fused with P3U1 myeloma cells. Hybrid cells were selected by culture in HAT medium 18 h after the fusion. Screening of the hybridoma supernatants was performed by a conventional ELISA assay using saporin-coated microtiter plates and a peroxidase-conjugated rabbit anti-mouse Ig as detection system (Perry & Kierkegaard). Three hybridomas, named CY12, CY56 and CY62 were selected according to this screening and were repeatedly subcloned by limiting dilution. All anti-saporin mAbs belonged to the IgG1 isotype and displayed a strong reactivity in ELISA assays with saporin without cross-reactivity with other RIPs. Culture medium for hybrids was D-MEM (Seromed) supplemented with 10% fetal calf serum, 1% bacterial penicillin/streptomycin, 100 U/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine.
with 10% FCS (Seromed) and 2 mM L-glutamine (Flow).

The MAR93 (IgG1) hybridoma produces an anti-CD25 mAb (Lopez Botet et al., 1986). Hypoxanthine-guanine phosphoribosyltransferase (HGPR) deficient mutants of the CY12.14 hybridoma were selected by culture in the presence of increasing amounts of 8-azaguanine (1 to 50 μg ml⁻¹) (Sigma, St. Louis, MO). Hybrid mutants were checked by limiting dilution and tested for HAT sensitivity and antibody production.

**Cell fusion and screening of hybrid hybridomas**

The HGPR-CY12.14 clone was fused with an idoacetamide-inactivated MAR93 hybridoma cells at a 1:1 ratio using a standard polyethylene glycol fusion protocol (Clark & Waldman, 1987; Suresh et al., 1986). Hybrid hybridomas were selected by culture in HAT medium. Culture supernatants were tested for their ability to react with saporin in ELISA assay and with CD25 + cells in an indirect immunofluorescence assay. Double-positive cultures were immediately subcloned by limiting dilution.

**BimAb purification**

Anti-saporin/anti-CD25 bimAb was purified from ascitic fluid by affinity chromatography on saporin coupled with Sepharose (Pharmacia). CNBr-activated sepharose (4 ml) was coupled with purified saporin (10 mg) previously dialysed against coupling buffer (NaHCO₃ 0.1 M pH 8.3, NaCl 0.5 M). Ascites was dialysed against 10 mM Tris (pH 7.5) and then incubated with saporin-sepharose for 1 h at 4°C. The elution of the bound material was performed by applying 100 mM glycine pH 4 and 100 mM glycine pH 2.5. The eluted fractions were collected in tubes containing an appropriate volume of 1 M Tris pH 8.0 to neutralise the pH. Reactivity of the purified bimAb with CD25 + targets was further checked by immunofluorescence, as described below.

**Cell lines**

Two different CD25 + cell lines have been used throughout the study: L540 (a kind gift of Dr A. Engert, Immunitoxin Labor, Medizinische Klinik, Kohn, Germany), derived from the lymph node of a Hodgkin’s lymphoma patient (Engert et al., 1992; ATL-derived MT2 (Miyoshi et al., 1981) (a kind gift of Prof. O. Varmier, Institute of Microbiology, University of Genova, Italy), derived from HTLV-I infected cells. A conventional Epstein Barr Virus (EBV)-transformed, CD25-negative B LCL, was used as an irrelevant target. All cells were maintained in RPMI 1640 medium containing 10% foetal bovine serum (FBS) (Gibco) and antibiotics (all reagents were from Seromed). Cells were used for assays during the logarithmic phase of growth. Viability was checked by trypan blue dye exclusion.

**Immunofluorescence staining**

The reactivity of bimAb was checked by indirect immunofluorescence on CD25-positive (L540, MT-2 and PHA-activated lymphoblasts) and CD25-negative cells (EBV-transformed B cell lines). Briefly, cells from the above cell lines were incubated with the bimAb anti-CD25, and with the two parental mAbs (anti CD25, MAR93, and anti-saporin, Cy12.14) for 30 min at 4°C. After two washes with cold phosphate buffered saline (Gibco) containing 2% of FBS (PBS-FBS) the cells were incubated for 30 min at 4°C with 5 μl of FITC-conjugated sheep anti-mouse-IgG immunoglobulins. After two washes with PBS-FBS, the samples were analysed by an EPICS cytomter. Appropriate Ig matched controls were run for each sample.

**Protein synthesis inhibition**

Cells (2 × 10⁸) were seeded in 96 well U-bottomed plates in a volume of 100 μl of complete RPMI 1640 medium. Saporin and bimAb were added to a final volume of 200 μl. Different concentrations of bimAb (from 10⁻⁸ to 10⁻¹³ M) were tested, in the presence of saporin (from 10⁻¹³ to 10⁻⁹ M) in order to titrate both reagents. Appropriate control samples were run with cells alone, saporin alone, parental mAbs alone, or saporin plus parental mAbs, mixed together. After 24 h of incubation 2 μCi of ³H-Leucine (Amersham) were added to each well. After additional 18 h the cells were harvested onto glass fiber filters by a cell harvester (ICN-Flow), and the radioactivity was evaluated by a β-computer (Packard), as already described (Tazzari et al., 1992a,b). Each experiment was run in triplicate. Mean values of five different experiments are expressed as percentage of control values. s.d. <15%.

**Inhibition of ³H-thymidine uptake by PHA-stimulated lymphocytes**

Normal T lymphocytes, upon activation, express the CD25 antigen. Thus we assayed the same combinations of saporin, bimAb, and parental mAbs as described above, on PHA-activated peripheral blood lymphocytes. Briefly, heparinised peripheral blood was obtained by venesection from five healthy volunteers, gradient-separated on a Ficoll-Hypaque cushion, counted and adjusted to a concentration of 5 × 10⁵ cells ml⁻¹ in complete RPMI 1640 medium containing 10% AB serum (Gibco). One hundred microliters of cell suspension (5 × 10⁶ cells) were then seeded in 96-well flat-bottomed plates (Falcon) in the presence of 5 μg ml⁻¹ of PHA (Sigma); 100 μl of different dilutions of saporin + bimAb, saporin alone, bimAb alone or appropriate mixtures of parental antibodies and saporin were added as described in the previous paragraph. After 48 h of incubation, ³H-thymidine (Amersham) (0.5 μCi per well) was added. After additional 24 h of incubation, cells were harvested onto glass fiber diskettes and processed as described above.

Each experiment was run in triplicate. Mean values of five different experiments are expressed as percentage of control values. s.d. <15%.

**Cell killing efficiency**

To evaluate the actual killing capacity, 2 × 10⁵ cells L540 cells were seeded in 24 wells plate in a final volume of 1 ml of complete RPMI 1640 medium, and incubated with different combinations of bimAb and saporin (see Table 1). Controls with saporin alone or bimAb alone were run. After 72 h of incubation at 37°C cell viability was evaluated using a standard trypan blue exclusion assay. A 72 h period of culture was selected for this assay on the basis of time course experiments, which indicated that this period of culture induced an optimal cell killing effect. Results were expressed as % of viable cells (mean values of three experiments run in triplicate, with s.d. <15%).

**Results**

**Characterisation of anti-saporin mAbs**

Three hybridomas secreting anti-saporin mAbs were produced by the fusion of immune mouse splenocytes with the

| Table 1 | Cytotoxic effect of bimAb in the presence of saporin on CD25 + tumour cells |
|---------|----------------------------------|
|         | 10⁻⁸ | 10⁻⁹ | 10⁻¹⁰ | No addition |
| Saporin (M) |       |      |       |            |
| 10⁻⁹ | 2%*  | 11%  | 27%   | 67%       |
| 10⁻⁸ | 9%   | 2%   | 65%   | 98%       |
| 10⁻⁹ | 69%  | 72%  | 78%   | 95%       |

*Data are expressed as % of viable L540 cells, evaluated by trypan blue exclusion.
P3U1 myeloma. All hybrids secreted IgGl mAbs which reacted with saporin in an ELISA assay, while they failed to react with other RIPS – momordin, gelonin, ricin-A chain, PAP-S and PAP-L. These results were confirmed by western blot analysis which showed that mAbs only reacted with the 30 kD saporin molecule (data not shown).

We next investigated whether the three mAbs could interfere with the ribosome-inactivating activity of saporin in a cell-free protein synthesis inhibition assay (Stirpe et al., 1983; Bolognesi et al., 1992). Two of them (CY56 and CY62) displayed > 80% of inhibition of the ribosome-inactivating activity, while the third (CY12.14) had virtually no inhibitory activity. To exclude the possibility of inhibitory effects on the ribosome-inactivating activity of saporin, the CY12.14 hybridoma was used for bimAb production.

Selection of a hybrid hybridoma producing anti-saporin/anti-CD25 bimAbs

To produce hybrid hybridomas secreting bimAbs a HGPRT-deficient CY12.14 anti-saporin hybridoma clone was fused with chemically inactivated cells of the anti-CD25 MAR93 hybridoma. After selection in HAT medium, hybrid hybridoma supernatants were screened for their ability to react simultaneously with saporin in ELISA and with CD25+ cells in immunofluorescence assays. Hybrids were repeatedly subcloned by limiting dilution and several subclones with double reactivity were selected. The supernatant of selected clones was then analysed for its ability to enhance saporin toxicity against CD25+ L540 target cells in a 3H-leucine uptake assay. The TC37 subclone displayed the highest activity in this assay, while the supernatant of other hybrid hybridoma subclones had less or no effect (data not shown).

This finding may be related to either chromosomal instability, leading to the loss of Ig genes, or to the secretion of different amounts of correctly assembled bispecific antibody compared to other possible combinations of secreted antibodies (Milstein & Cuello, 1984). BimAb was then purified from TC37 hybrid hybridoma ascites by affinity chromatography on a saporin-Sepharose column and utilised for further studies.

**BimAb reactivity**

The CD25 + L540 and MT2 cell lines strongly reacted with the bimAb or with the parental anti-CD25 mAb as assayed by immunofluorescence, whereas they did not react with the anti-saporin CY12.14 mAb (Figure 1). In addition the bimAb reacted with 20–30% of 48 h PHA-stimulated normal lymphocytes, but not with a CD25-negative (EBV)-transformed B LCL (data not shown). Thus the bimAb recognising both CD25 antigen and saporin maintains the capability of selectively recognising CD25+ cells.

**Effect of bimAb-saporin combinations on protein synthesis inhibition**

Titration experiments using serial concentrations of both bimAb and saporin were performed with the L540 and MT2 CD25+ cell lines. As shown in Figure 2b protein synthesis by L540 cell line was inhibited by saporin with an IC50 (concentrations giving 50% inhibition in comparison to control values) of 8 x 10^-12 M, in the presence of 10^-9 M of bimAb, which was the optimal concentration for enhancing saporin toxicity. In the presence of 10^-8 M of bimAb saporin had an IC50 of 5 x 10^-11 M, suggesting that the bimAb was in excess in comparison to the capacity of cell binding sites, thus preventing saporin internalisation. An IC50 of 2.5 x 10^-11 M (as saporin) was obtained in the presence of a concentration of 10^-10 M of bimAb. BimAb concentrations of 10^-11 M, 10^-12 M and 10^-13 M were effective with an IC50 of 3 x 10^-10 M, 7 x 10^-10 M and 9 x 10^-10 M, respectively. The bimAb alone did not inhibit protein synthesis. Saporin alone had an IC50 of 4 x 10^-9 M (Figure 2a), which was not enhanced in the presence of the parental antibody against saporin (CY12.14), and was virtually unchanged by the mixture of the two parental mAbs (anti-CD25 and anti-saporin mAbs) (Figure 2a).

Similar results were obtained using the ATL-derived MT2 cell line. In particular maximum inhibition of protein synthesis was obtained at concentrations of 10^-8 M and 10^-10 M of bimAb (saporin IC50s were 3 x 10^-11 M and 1.3 x 10^-10 M respectively, Figure 3b). At a bimAb concentration of 10^-8 M

**Figure 1** Cytofluorimetric analysis of anti-saporin/anti CD25 bimAb reactivity on the CD25+ MT2 cell line by indirect immunofluorescence.
Figure 2. Inhibition of protein synthesis by the L540 target cells treated with different bimAb/saporin combinations. Experiments performed as described in Materials and methods section. s.d. <15% a, as experimental controls, L540 cells were treated with: saporin alone –Δ–, anti-CD25 mAb alone –+–, anti-saporin mAb alone –O–, bimAb alone –□–, anti-saporin mAb + saporin –X–, anti-CD25 mAb + saporin –Δ–, anti-saporin mAb + anti-CD25 mAb + saporin –■–. b, L540 cells were treated with different concentrations of bimAb in the presence of serial dilutions of saporin (10⁻³ to 10⁻¹³ M). BimAb concentrations: 10⁻⁷ M –□–; 10⁻⁹ M –O–; 10⁻¹⁰ M –Δ–; 10⁻¹¹ M –X–; 10⁻¹² M –■–; 10⁻¹³ M –□–.

Figure 3. Inhibition of protein synthesis by the MT2 target cells treated with different bimAb/saporin combinations. Experiments performed as described in Materials and methods section. s.d. <15% a, as experimental controls, MT2 cells were treated with: saporin alone –Δ–, anti-CD25 mAb alone –+–, anti-saporin mAb alone –O–, bimAb alone –□–, anti-saporin mAb + saporin –X–, anti-CD25 mAb + saporin –Δ–, anti-saporin mAb + anti-CD25 mAb + saporin –■–. b, MT2 cells were treated with different concentrations of bimAb in the presence of serial dilutions of saporin (10⁻³ to 10⁻¹³ M). BimAb concentrations: 10⁻⁷ M –□–; 10⁻⁹ M –O–; 10⁻¹⁰ M –Δ–; 10⁻¹¹ M –X–; 10⁻¹² M –■–; 10⁻¹³ M –□–.

A lower degree of inhibition was observed (IC₅₀ of 1.5 x 10⁻¹⁰ M as saporin), as already observed for the L540 cell line. Concentrations of bimAb ranging from 10⁻¹¹ M to 10⁻¹⁰ M gave saporin IC₅₀s ranging from 8 x 10⁻¹⁰ M to 9 x 10⁻¹⁰ M. Different mixtures of parental antibodies and saporin did not specifically affect protein synthesis by MT2 cells (Figure 3a).

A CD25-negative EBV-transformed LCL was used as an irrelevant target in a [³H]-leucine uptake assay. No enhancement of saporin specific toxicity was observed by testing all the combinations of bimAb and saporin, as described above (data not shown).

Cytotoxic effects of bimAb-saporin combination

To verify that cells were indeed killed by bimAb-saporin combination, we evaluated cell viability after 48 h of incubation. Trypan blue-dye exclusion assay demonstrated that L540 cells were efficiently killed (only 2–11% of viable cells) at concentrations of bimAb ranging from 10⁻⁸ M to 10⁻⁹ M in the presence of saporin concentrations in the same range. Under the same conditions saporin alone induced only a partial loss of viability at a concentration of 10⁻⁸ M (67% of viable cells) (Table I).

Inhibition of [³H]-thymidine incorporation on PHA-stimulated lymphocytes

Since the CD25 antigen is expressed on normal T-lymphocytes upon activation, we investigated whether combinations of bimAb and saporin were effective in blocking [³H]-thymidine incorporation in PHA-stimulated lymphocytes. Our experiments demonstrate that, in the presence of bimAb ranging from 10⁻⁸ M to 10⁻⁹ M, saporin had IC₅₀s of 10⁻¹¹ M, 3.8 x 10⁻¹¹ M and 6 x 10⁻¹¹ M respectively (Figure 4b). Lower concentrations of bimAb (from 10⁻¹¹ M to 10⁻¹³ M) resulted in IC₅₀s of 2 x 10⁻¹⁰ M, 5.5 x 10⁻¹⁰ M, and 2 x 10⁻⁹ M respectively (Figure 4b). In the presence of a mixture of both parental mAbs, saporin showed an IC₅₀ of 4 x 10⁻⁹ M. Parental mAbs had no toxicity, while saporin alone had an IC₅₀ of 10⁻⁸ M (Figure 4a).

altogether, these results indicate that the anti-CD25/anti-saporin bimAb is able to efficiently target the toxic activity of saporin against both normal and neoplastic CD25 + lymphoid cells.

Discussion

In this study we described a bimAb recognising both the CD25 antigen and saporin, one of the most powerful RIPS type 1 (Stirpe et al., 1983; Thorpe et al., 1985; Stirpe et al., 1992; Bolognesi et al., 1992). The bimAb enhanced saporin toxicity against both normal and neoplastic CD25 + lymphoid cells lowering saporin IC₅₀ to about 10⁻¹¹ M in the presence of 10⁻⁹ M and 10⁻¹⁰ M bimAb. Irrelevant CD25-negative target cells were unaffected, thus confirming the specificity of the bimAb targeting activity.

These data were obtained by the evaluation of the inhibitory effect on protein and DNA synthesis. Indeed the bimAb-toxin mixture had cytotoxic effects as demonstrated by a loss of cell viability, even though the latter assay shows...
usually a lower degree of sensitivity (Tazzari et al., 1992a,b). The ability of the bimAb to enhance saporin toxicity was strictly dependent on its double specificity, since parental (anti-saporin or anti-CD25) mAbs, either alone or in combination, were ineffective. It should be pointed out that the double specificity of the bimAb was demonstrated by its ability to recognize CD25 + target cells after the affinity chromatography purification on saporin-Sepharose. This purification procedure allowed the removal of antibody species not reacting with saporin (such as parental anti-CD25 mAb) secreted by the hybrid hybridoma, while parental anti-saporin mAb co-eluted together with bimAb. Therefore, the complete removal of other antibody species (such as parental anti-saporin mAb) from bimAb preparations, by the use of additional purification steps, may result in an enhancement of bimAb targeting activity.

One of the basic conditions for a possible in vivo targeting is the reactivity of the reagent of choice with a restricted cell population, excluding vital tissues. The CD25 antigen could be an optimal choice, since in normal healthy individuals it is expressed only on small fractions of activated B- and T-lymphocytes (Herve' et al., 1990). The CD25 molecule represents the α-chain of the interleukin-2 (IL-2) receptor complex. This chain, together with the IL-2 receptor β chain, forms the high affinity IL-2 receptor which is expressed on both T- and B-lymphocytes after activation (Dukovich et al., 1987; Robb et al., 1987). Thus CD25 plays a key role in the control of IL-2-driven T and B lymphocyte proliferation and in the clonal expansion of antigen-specific lymphocytes. Therefore the possible clinical applications of anti-CD25 mAbs may be the targeting of CD25 + lymphoid cell tumours, or of CD25 + normal cells in severe immune disorders such as GVHD and transplanted organ rejection.

In a previous study we described an immunotoxin obtained by conventional chemical linking of a CD25 mAb and saporin, with a very high specific in vitro activity (IC₅₀ of approximately 10⁻¹⁰ M as linked saporin), which was designed for the therapy of steroid-resistant GVHD, as well as of CD25 + lymphomas (Tazzari et al., 1992b). Moreover our group recently reported in vitro and in vivo results obtained in CD30 + lymphoid neoplasias with an immuno-toxin containing an anti-CD30 mAb (Ber-H2) chemically linked to saporin (Tazzari et al., 1992a; Falini et al., 1992). Encouraging clinical results confirmed the high activity of saporin-containing conjugates demonstrated in the in vitro assays (Thorpe et al., 1985; Siena et al., 1988; Tazzari et al., 1988). The major limitations were represented by (i) the higher toxicity of linked saporin in comparison to free saporin (LD₅₀ in mice 1 mg kg⁻¹ vs 10 mg kg⁻¹ (Thorpe et al., 1985; Marcucci et al., 1989)) and (ii) the formation of anti-saporin and anti-mouse IgG antibodies, with increased risk of anaphylaxis due to a possible enhanced immunogenicity of linked saporin (Falini et al., 1992; F. Stirpe, personal observations). Thus the possible advantages of the in vivo use of bispecific antibodies recognising both the relevant target and saporin are (i) the possibility of in vivo targeting with higher doses of mAb and saporin; (ii) a lower probability of anaphylaxis after repeated administrations and (iii) to avoid the partial and in some cases (gelonin) almost total inactivation of RIs which may occur during the chemical conjugation to antibodies (Bolognesi et al., 1992).

Immunotoxins consisting of mAbs and toxic moieties may provide efficient tools to reduce the toxicities of conventional chemotherapeutic and immunosuppressive protocols. The bimAb herein described could provide the basis for a clinical trial, devised to effectively target and kill CD25 + unwanted populations, both normal and of neoplastic origin. In this context bispecific antibodies recognising saporin and B-cell antigens have recently been reported and were used in a clinical trial in patients with CD22 + low grade lymphomas, with encouraging clinical results (Bonardi et al., 1992).

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References

BARBIERI, L., STOPPA, C. & BOLOGNESI, A. (1987). Large scale chromatographic purification of ribosome-inactivating proteins. J. Chromatogr., 408, 235–243.

BOLOGNESI, A., TAZZARI, P.L., TASSI, C., GROMO,G., GOBBLIM. & STIRPE, F. (1992). A comparison of anti-lymphocyte immunotoxins containing different ribosome-inactivating proteins and antibodies. Clin. Exp. Immunol., (in press).

BONARDI, A.M., BELL, A., FRENCH, R.R., GROMO, G., HAMBLIN, T., MODENA, M., TUTT, A.L. & GLENNIE, M.J.(1992). Initial experience in treating human lymphoma with a combination of bispecific antibody and saporin. Int. J. Cancer Suppl., 7, 73–77.

CLARK, M.R. & WALDMANN, H. (1987). T-cell killing of target cells induced by hybrid antibodies: comparison of two bispecic monoclonal antibodies. J.NCI, 79, 1393–1401.

DUKOVICH, M., WANO, Y., BICH THUY, L-T., KATZ, P., CULLENS, B., KEHR, J.H. & GREENE, W.C. (1987). A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors. Nature, 327, 518–521.
ENGERT, A., BURROWS, F., JUNG, W., TAZZARI, P.L., STEIN, H., PFREUNDSCHU, M., DIEHL, V. & THORPE, P. (1990). Evaluation of ricin A chain-containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. Cancer Res., 50, 84–88.

FALINI, B., BOLOGNESI, A., FLENGHI, L., TAZZARI, P.L., BROE, M.K., STEIN, H., DURKOP, H., AVERSA, F., CORNELI, P., PIZZI, G., BARBADOLEGA, G., SABBATTINI, E., PILERI, S., MARTELLI, M.F. & STIRPE, F. (1991). Response of refractory Hodgkin's disease to therapy with anti CD30 monoclonal antibody linked to saporin (Ber-H2/S06) immunotoxin. Lancet, 339, 1195–1196.

FRANKEL, A.E. (ed) (1988). Immunotoxins. Kluwer Academic Publishers.

FERRINI, S., PRIGIONE, I., MAMMOLITI, S., COLNAGHI, M.I., MENARD, S., MORETTA, A. & MORETTA, L. (1989). Re-targeting of human monoclonal antibodies expressing the T-cell receptor gamma/delta to ovarian carcinoma cells by the use of bispecific monoclonal antibodies. Int. J. Cancer, 44, 245–250.

FERRINI, S., PRIGIONE, I., MIOTTI, S., CICCONE, E., CANTONI, C., CHEN, Q., COLNAGHI, M.I. & MORETTA, L. (1991). Bispecific monoclonal antibodies directed to CD16 and to a tumor-associated antigen induce target-cell lysis by resting NK cells and by a subset of NK clones. Int. J. Cancer, 48, 227–233.

GLENNIE, M., BRENNAND, D., BRYDEN, F., MCBRIDE, H., STIRPE, F., WORTH, A. & STEVENSON, G. (1988). Bispecific F(ab')2 antibody to deliver saporin in the treatment of lymphoma. J. Immunol., 141, 3662–3670.

HERVE, P., WIJDENES, J., BERGERAT, J.P., BORDIGONI, P., MILPIED, N., CAHN, J.Y., RACADOT, E., GAUD, C., BELIARD, D., TROGUSRAD, X. & BENZ-LEMOINE, E. (1990). Treatment of corticosteroid-resistant acute graft-versus-host disease by in vivo administration of anti-IL2 receptor monoclonal antibody (B10). Blood, 75, 1017–1023.

LAKY, M., MOTA, G., MORARU, I. & GHETIE, V. (1987). Binding and cytotoxic effect of ricin toxin on multivalent hybrid antibody-coated target cells. Immunol. Lett., 14, 127–132.

LANZAVECCIA, A. & SCHEIDDEGGER, N. (1987). The use of hybrid hybridomas to target human cytotoxic T lymphocytes. Eur. J. Immunol., 17, 105–111.

LOPEZ BOTE, M., MORETTA, A., LOWENTHAL, J., ACCOLLA, R., PANTALEO, G. & MORETTA, L. (1986). Characterization of monoclonal antibodies directed against the human interleukin-2 receptor. Immunologia, 5, 46–55.

MARCUCI, F., LAPPI, D.A., GHISSLIERI, M., MARTINEAU, D., FORMOSA, A., SIENA, S., BREGNI, M., STIRPE, F. & GIANNI, A.M. (1989). In vivo effects in mice of an anti-T cell immunotoxin. J. Immunol., 142, 2951–2960.

MILSTEIN, C. & CUELLO, A.C. (1984). Hybrid hybridomas and the production of bispecific monoclonal antibodies. Immunol. Today, 5, 299–304.

MYOSHI, I., KUBONISHI, I., YOSHIMOTO, S., AKAGI, T., OHTSUKI, Y., SHIRAISHI, Y., NAGATA, K. & HINUMA, Y. (1981). Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukemic T cells. Nature, 294, 770–771.

ROBB, R.J., RUSK, C.M., YODOI, J. & GREENE, W.C. (1987). Interleukin-2 binding molecule high affinity receptor distinct from Tac protein: analysis of its role in formation of high-affinity receptors. Proc. Natl Acad. Sci. USA, 84, 2002–2007.

SIENA, S., LAPPI, D.A., BREGNI, M., FORMOSA, A., VILLA, S., SORIA, M., BONADONNA, G. & GIANNI, A.M. (1988). Synthesis and characterization of an antihuman T-lymphocyte saporin immunotoxin (OKT3-SAP) with in vivo stability into nonhuman primates. Blood, 72, 756–765.

STAERZ, U.D. & BEVAN, M.J. (1986). Hybrid hybridoma producing a bispecific monoclonal antibody that can focus effector T-cell activity. Proc. Natl Acad. Sci. USA, 83, 1453–1457.

STIRPE, F., BARBIERI, L., BATTELLI, M.G., SORIA, M. & LAPPI, D.A. (1992). Ribosome-inactivating protein from plants: present status and future prospects. BioTechnol., 10, 451–462.

STIRPE, F., GASPERI-CAMPAINI, A., BARBIERI, L., FALASCA, A., ABBONDANZA, A. & STEVENS, W.A. (1983). Ribosome-inactivating proteins from the seeds of Saponaria officinalis (soapwort), of Agrostemma githago L. (corn cockle) and Asperagus officinalis L, and from the latex of Hura crepitans L (sandbox tree). Biochem. J., 216, 617–625.

SURESH, M.R., CUELLO, A.C. & MILSTEIN, C. (1986). Bispecific monoclonal antibodies from hybrid hybridomas. Methods Enzymol., 121, 210–228.

TAZZARI, P.L., BARBIERI, L., GOBBI, M., DI NOTA, A., RIZZI, S., BONFADINI, A., PESSION, A., TURA, S. & STIRPE, F. (1988). An immunotoxin containing a rat IgM antibody Campath 1 and saporin 6: effect on T lymphocytes and hemopoietic cells. Cancer Immunol. Immunother., 26, 231–236.

TAZZARI, P.L., BOLOGNESI, A., DE TOTERO, D., FALINI, B., LEMOLI, R.M., SORIA, M.R., PILERI, S., GOBBI, M., STEIN, H., FLENGHI, L., MARTELLI, M.F. & STIRPE, F. (1992a). Ber-H2 (anti-CD30)-saporin immunotoxin: a new tool for the treatment of Hodgkin's disease and CD30+ lymphoma: in vitro evaluation. Br. J. Haematol., 81, 203–211.

TAZZARI, P.L., BOLOGNESI, A., DE TOTERO, D., PILERI, S., CONTE, R., WIJDENES, J., HERVE, P., SORIA, M. & STIRPE, F. (1992b). B-B10 (anti-CD25)-saporin immunotoxin. A possible tool in graft-versus-host disease treatment. Transplantation, 54, 351–356.

THORPE, P.E., BROWN, A.N.F., BREMNER, J.A.G., FOXWELL, B.M. & STIRPE, F. (1985). An immunotoxin comprising a monoclonal antibody anti-THY 1.1 antibody and a ribosome-inactivating protein from Saponaria officinalis: potent antitumor effects in vitro and in vivo. J. Natl Cancer Inst., 19, 157–162.