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Perspectives and Commentaries

Removal of Tumour Cells from Bone Marrow: Overview

D. BRON and P. STRYCKMANS

Service de Médecine Interne et Laboratoire d'Investigation Clinique H. J. Tagnon, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, 1 rue Héger Bordet, 1000 Bruxelles, Belgium

(A COMMENT ON: Bernard J, Bettan-Renaud L, Gavoille A *et al. In vitro* chemical eradication of small cell lung cancer: application in autologous bone marrow transplantation. *Eur J Cancer Clin Oncol* 1988, **24**, 1561–1566.)

INTRODUCTION

AUTOLOGOUS bone marrow transplantation has offered a way of increasing the dose of drugs and radiotherapy in an attempt to cure patients with malignant disease. However, bone marrow may be contaminated with tumour cells at the time of harvest and it is undesirable to return these to the patients. Little data are available concerning the number of tumour cells necessary to reseed various malignancies and this number is probably different for each tumour [1]. Also, the immunodeficiency of the patient after intensive chemo and/or radiation therapy may favour the growth of a malignant clone.

Therefore, purging of the marrow is usually considered as a useful procedure and a variety of properties distinguishing the malignant cells from the normal marrow cells have been exploited to eliminate the undesirable cells. These methods include immunological, chemical and physical approaches.

IMMUNOLOGICAL APPROACH

Following the development of monoclonal antibodies (MoAb) major advances have been achieved and mostly applied to the purging of marrow from leukaemic patients. Due to the heterogeneity of leukaemic cells, a combination of MoAb was more effective than one MoAb at killing tumour cells [2]. The purge of the marrow with MoAb is no longer restricted to haemopoietic malignancies and several MoAb directed against antigens present on tumour cells and not on haematopoietic progenitor cells (HPC) have been investigated. For instance, Fib-75 recognizing an antigen present on epithelial tumour cell but not on HPC, has been used to purge marrow from breast, bladder and lung cancer cells [3]. Other interesting antibodies are directed against neuroblastoma [4]. Several of these react with immature B-cells but none of these react with HPC and therefore are now widely used for removing neuroblastoma cell from autologous bone marrow graft. A substantial problem remains associated with monoclonal antibodies and complement-mediated lysis from the fact that murine MoAb usually do not fix human complement and that only IgM and IgG 2b subclasses seem quite effective in fixing rabbit or guinea pig complement. In addition, the inherent batch to batch variability of these reagents for both lytic activity and toxicity implies multiple screening before use.

To circumvent the necessity of using heterogeneic complement, monoclonal antibodies have been conjugated with various toxins. The toxin receiving the most attention is ricin and antibody–ricin conjugates are often referred to as immunotoxins [5]. Unfortunately, lysis with immunotoxins taking hours or days, this procedure is considerably longer than complement-mediated lysis and the marrow can be damaged by a too long *ex vivo* incubation. Other drugs have been coupled to an antibody (isocyanate derivative of chlorambucil, vindesine) and proved to have a

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selective toxicity to the tumour cell. However, a variability in terms of killing activity was observed between conjugates and the reasons for these variations remain unclear. Antibody-targeted liposomes containing drug were found to be 55 times more effective in killing K562 cells than non-targeted liposomes [6], but to ensure an optimal killing, up to 48 h incubation is necessary and, again, the viability of the HPC may be affected.

A theoretical problem associated with the killing of malignant cells is the release of transforming DNA material generated by the lysis of the tumour cell. The immunomagnetic approach recently developed by Kemshead *et al.* [4] and successfully applied to remove neuroblastoma cells from marrow, avoids this undesirable effect. This technique consists of using monoclonal antibodies to target magnetic compounds (polystyrene beads containing magnetite) to tumour cells and separating the bead-coated tumour cells from non-malignant cells without lysis of the cell, by high magnetic field separation. This system, using a panel of six antibodies against neuroblastoma cells, allows a 3 log depletion of tumour cells without altering the quality of marrow progenitors and can be applied to other malignancies involving the marrow. In a similar approach, we and others [7, 8] have developed an immunomagnetic procedure using iron or cobalt magnetic colloid coupled with an anti-CALLA monoclonal antibody. Direct conjugation of MoAb can be performed or indirect labeling can be used via a particle-conjugated anti-mouse immunoglobulin. The latter technique is easier, allowing the use of one batch of conjugated second antibody with different MoAbs but in our experience, its purging efficiency is lower than that of the direct procedure. Besides, being more effective with monoclonal antibodies of any Ig subclasses, immunomagnetic purging requires smaller amounts of antibodies, is more rapid and easier to handle.

NON-IMMUNOLOGICAL APPROACH

Lectins such as soybean agglutinin and peanut agglutinin were first proposed to remove T-cell from allogeneic bone marrow in order to reduce the incidence of graft-versus-host disease. This work was extended by using soybean agglutinin for removing human leukaemic and neuroblastoma cell lines but there is considerable heterogeneity in the binding of neuroblastoma cells. This fact severely limits its use for marrow purging.

Among the cytotoxic drugs used to purge bone marrow, 4-hydroperoxycyclophosphamide (4HC) and hydroxazaphosphorine or mafosfamide (AZ) have certainly gained the widest enthusiasm for leukaemic and lymphoma patients [9]. This approach has been compared with a cocktail of monoclonal antibodies in cleansing clonogenic Burkitt's lymphoma cells from human bone marrow and found to have comparable efficacy. Also, the combination of both treatments was shown to increase the antitumour activity [10].

In another issue of this journal, Bernard *et al.* [11] investigated the ability of several compounds (doxorubicin, *cis*-platinum, ditercalinum, celiptium, VP16-213, AZ) to eradicate *in vitro* small cell lung cancer (SCLC) cells from a mixture of normal human marrow and SCLC cells. They conclude that doxorubicin and ditercalinum, a new bis intercalator, are most efficient. *Cis*-platinum, however, gave the best therapeutic index because of its low myelotoxicity. According to their criteria, to preserve 50% of myeloid progenitors, no more than 10 μM of *cis*-platinum can be used, to obtain a 2 log depletion which is inferior to immunological procedures. It should be emphasized that none of the marrow culture systems used in Bernard's paper can now be considered as optimal to reflect the sensitivity of the pluripotent stem cell.

Tumour necrosis factor (TNF-α), a product of activated macrophage with cytotoxic activity on tumour cells, has been tested for its ability to purge marrow without damaging the quality of the marrow. One study demonstrates that haemopoietic precursors survive treatment with TNF-α at doses that have been shown to be cytotoxic to tumour cells (10,000 U/ml) [12].

High concentrations (ranging from 20 to 100 mEq/l) of lithium carbonate have also been shown in our laboratory to be more cytotoxic to clonogenic leukaemia cells from patients with acute non-lymphoblastic leukaemia in the acute phase than to HPC *in vitro* [13]. The mechanism for this specific killing of leukaemic progenitors still remains unclear. In fact, it seems to be related to the lithium itself but also to the increase of pH induced by the carbonate moiety of the molecule.

Another interesting approach has been described by Atzpodien *et al.* [14] using merocyanin-540 (MC-540), a fluorescent dye. MC-40 preferentially fixes the leukaemic cell membrane and, after light exposure, can induce photolysis of the cell. Using a model of normal human marrow contaminated with HL-60 leukaemic cells, a 4 log depletion was obtained for tumour cells versus 1 log for HPC. MC-540, a differentiation marker on the haemopoietic stem cell [14] promises to be very useful alone or, in combination with other techniques, in the removal of leukaemic cells from bone marrow.

QUALITY CONTROL OF THE PURGING

Human marrow myeloid (CFU-GM) and erythroid (BFU-E and CFU-E) precursors are most frequently used to determine the best thera-

peutic index (highest anti-tumour activity with lowest myelotoxicity) of several compounds. The experience with 4HC has demonstrated that marrow engraftment can occur in the absence of CFU-GM growth [15] and even in the absence of CFU-GEMM, a multi-lineage marrow precursor [16] meaning that a more primitive progenitor is spared at 100 μg/ml of 4HC. Interestingly, Gordon *et al.* [17] have developed an *in vitro* culture model of primitive progenitor colonies (PPC) using, in a first step, a human marrow feeder layer of adherent fibroblasts and fat bone marrow cells and, in a second step, a culture of marrow stem cells which have the property to attach to this feeder layer. Using this culture model, both VP16-213 and 4HC were shown to have specific toxicity for tumour cells which was contrasting with lack of specificity when using the CFU-GM and BFU-E [17, 18].

Another limitation to control the quality of the marrow purging is the absence so far of a satisfying method to detect one tumour cell in 10^9 to 10^{10} nucleated marrow cells.

CONCLUDING REMARKS

The clinical feasibility of several purging procedures described in this overview has been demonstrated. A major uncertainty, however, concerns their clinical usefulness.

Although a high performance of purging is obtained using marrow contaminated with tumour cells from established cell lines, these experimental models do not reflect what happens with fresh tumour cells which show considerable heterogeneity of antigen expression. In the setting of immunological methods, the possibility exists that monoclonal antibodies could recognize only the most differentiated cells—leukaemia illustrates it very well—and none or few of the leukaemic stem cells. This limitation could affect differently the immunological and non-immunological approaches. It should be stressed also that, so far, no controlled study has ever demonstrated the superiority of purged over non-purged marrow in autologous bone marrow transplantation, and randomized clinical trials are urgently needed to bring an answer to these important questions.

In the future, an interesting approach will be to remove an early HPC from patients' bone marrow for subsequent autografting (positive separation) but the appropriate MoAb directed at antigens of the early HPC, and not present on the tumour cell are not yet available.

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