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Autologous Bone Marrow Transplantation in Hematological Malignancies*

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AUTOLOGOUS bone marrow transplantation is a new technique which allows some hope for the treatment of certain hematological malignancies and a few solid tumors. It consists of engrafting a patient, after ablative chemotherapy and/or whole-body radiation, with his own marrow, previously collected at a propitious moment in the history of the disease. This technique has 2 consequences of major interest:

(1) Autotransplantation by reducing the length and variability of post-treatment aplasia allows the use of chemotherapeutic agents at high doses which greatly surpass the threshold of myelotoxicity. The higher doses of treatment can result in a greater tumor kill, an effect which was impossible to study in the past.

(2) The marrow of patients with acute leukemia (AL) can be collected during complete remission (CR) and cryopreserved. This marrow can later, at the time of relapse, be reinfused after high-dose chemotherapy and/or whole-body irradiation (TBI) to achieve another complete remission. This situation is a sort of chronological chimera in which the autograft, with younger stem cells than the organism would actually possess, reproduces, over the course of a few months or years, the evolution of the remission during which it was collected. In a more aggressive setting the same autograft can be used immediately, without waiting for a relapse, to allow ablative therapy in the consolidation mode. In both settings the technique is modeled on that of *allo*grafting and the conditioning regimen is actually identical to that which precedes an allograft. Recently techniques for purging the marrow of residual leukemic cells have been developed. It is conceivable that complete 'purification' will be possible in the near future. Hence autologous bone marrow transplantation may well appear as a plausible treatment for 75% of those patients with acute leukemia who, in the absence of a compatible donor or due to their age, cannot be considered for allogeneic marrow transplantation.

The situation is in some ways similar for chronic myelocytic leukemia (CML), where stem cells collected and cryopreserved during the chronic phase may be used later in an attempt to reverse the blast crisis and return to the chronic phase. However, the difficulties encountered in this last indication remain considerable.

We therefore propose to summarize the recent developments which now make autologous bone marrow transplantation (ABMT) practicable, the principal clinical results at the present time and the major steps of research, which will be grouped under four main headings: (1) acute leukemias, (2) chronic myelogenous leukemia, (3) non-Hodgkin's lymphoma and (4) solid tumors.

TECHNICAL CONSIDERATIONS

The freezing techniques for hematopoietic stem cells can now be considered to be adequate. They are all based on the same well-established principles: (a) use of a diffusable cryoprotector, such as dimethylsulfoxide (10% concentration); (b) addition of compatible human serum (5–10% concentration); (c) low concentration of the cells to be frozen; (d) freezing in homogeneous thin layers which favor thermal exchange; (e) use of

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non-lipophilic (polyolefine or Teflon–Capton) bags instead of plastic; (f) use of programmed freezers which ensure a slow thermal decrease (1–2°C/min) and a specific abolition of the heat of fusion; and (g) ultra-rapid thawing of the frozen marrow for immediate infusion [1–6].

Under these conditions the recovery of CFU-c is in the range of 75–100% for the marrows from patients with solid tumors or acute leukemias in CR [7–9]. Nevertheless, the reliability of the current cryopreservation techniques should not let one forget the exquisite fragility of the hematopoietic stem cells: recently we have drawn attention to the fact that an inadvertent increase in the rate of cooling after the release of the heat of fusion, which may occur from an over-admission of liquid nitrogen during the transition phase in an effort to shorten it, may lead to a massive destruction of the stem cells [10]. Also, the state within the cycle of mitosis of the hematopoietic stem cells undergoing freezing influences their resistance. Cells in G_0 are thus more fragile [11]. Finally, we have observed a poor recovery of CFU-c from patients with CML when the concentration is greater than 6500 CFUc/ml.

Usually, in adult patients a total volume of 600–1000 ml of marrow is removed in a surgery room under general anesthesia. This marrow can be frozen in its own plasma, but the red cells are lysed during thawing [5]. In many institutions the marrow is concentrated to its buffy coat on a cell separator. This technique allows a 85% recovery of total CFU-c in about 15% of the original volume [12]. More recently a technique of filtration over a Ficoll gradient was developed which enables the recuperation of a nearly pure mononuclear population containing almost 100% of the CFU-c in only 10% of the original volume [13, 14]. These methods save significant space in storage but their main interest is that they are a prerequisite for the *in vitro* therapy of the marrow, in particular with monoclonal antibodies. Until now stem cells collected and cryopreserved by these methods have produced autologous engraftment in apparently satisfactory conditions. However, at present there is insufficient data to compare the kinetics of recovery, haemopoiesis and immune functions in unmanipulated, centrifuged and filtered marrow. In the absence of more precise information, it is appropriate to remember that multiple relationships exist between the diverse populations that constitute the heterogeneous marrow [15, 16], which may point to the choice of a non-fractionated marrow for autograft whenever possible. Likewise, any manipulation of the marrow carries the risk of altering the stem cells qualitatively and quantitatively. Finally, if the marrow is concentrated, the concentration of the stem cells and nucleated cells should not reach such a level that mechanical damages intervene in the course of freezing or that aggregates appear during thawing [17].

The kinetics of recovery of haematopoiesis after ABMT are well known. In patients with non-Hodgkin's lymphoma treated with combination chemotherapy such as BACT (BCNU, cytosine arabinoside, cyclophosphamide and 6-thioguanine) [18] or TACC (CCNU instead of BCNU) [19], the median days of recovery to 1000 leucocytes/mm^3 and 50,000 platelets are days 12 (range 9–19) and 14 (range 8–27) respectively. The duration of aplasia under these conditions is less than a half of that observed following the same treatment without autotransplant. The risk of infection is slight and the platelet support is much easier (median number of platelet transfusions: 5 [20] to 9 [18]) than in the control group (median number: 14 [18]; $P < 0.05$).

The kinetics of recovery are slower in patients with acute leukemia [20], in patients with CML engrafted with stem cells collected in the peripheral blood [21] and following whole-body irradiation. In this last case the recovery of the neutrophiles and platelets occurs on days 20–35 and 23–34 respectively [22]. There is a direct relationship between the rate of hematopoietic recovery and the concentration of CFU-c in the administered marrow [23]. Failure of the autologous marrow to engraft may occur but is a rare event. In our own experience we have encountered a few often significant delays in platelet recovery [10]. It seems that, outside a few specific clinical situations, this can often be due to the *in vitro* manipulations of the marrow and/or to the particular sensitivity of megakaryocytes and their precursors to cryopreservation [24]. Similar prolonged thrombocytopenia has also been observed in the course of allogeneic marrow transplant.

Until now immunological recovery has not been studied extensively. We have shown [25] that the level of serum IgG decreases moderately but remains within normal limits for a transitory period of less than 2 months. There is a greater and longer decrease in IgA and IgM. In 2 cases we have been able to observe a primary immunization with initial production of IgM antibodies (1 anti-cytomegalovirus, 1 anti-B-K virus) followed by a rise in IgG antibodies. The lymphocyte response to mitogens (PHA, Con A, pokeweed, MLC) is temporarily depressed and returns to pre-transplant levels by day 200. It has also been shown recently by several teams that the ratio of helper/suppressor lymphocytes (OKT4/OKT8) is decreased following ABMT in a similar manner

as following allogeneic transplantation [26–28]. There seems to be a link between the dose of marrow infused and the rate of recovery of immune functions [25].

The risk of infectious complications following ABMT after the end of the aplastic phase is very low compared to the high risk persisting after allogeneic transplantation. Out of a total of 55 ABMT since 1976, we have seen only one fatal infection due to cytomegalovirus and one infection probably due to *Pneumocystis carinii* which resolved with appropriate therapy. Although more extensive immunological studies would be welcome, the comparison between auto- and allo-transplantations confirms the significant role of graft-vs-host reaction in the persistence of immunodepression and the risk of infection following allogeneic transplantation.

In conclusion, the risk of ABMT itself is small and, based on our own experience, ABMT has become a routine procedure provided that certain precautions be taken: collection of a sufficiently rich marrow (>1000 CFU-c/ml); CFU-c recovery >50% after thawing in test bags and in the bags of marrow administered to the patient; and a total dose of marrow >800 CFU-c/kg.

When these conditions are met the main risk actually lies in the extra-medullary toxicity of the pre-graft conditioning regimen, which includes irreversible cardiotoxicity of anthracyclines, cyclophosphamide, BACT, TACC [29] and radiation, and veno-occlusive disease of the liver [30, 31].

ACUTE LEUKEMIA

In the initial attempts the bone marrow was collected from adult patients in complete remission at variable points in the evolution of their disease and reinjected after whole-body radiation [32] or high-dose chemotherapy (such as TACC) [33] at the time of relapse. The complete remission rates were 55 and 78% respectively.

The best results (71 [32] and 90% [33, 34]) were obtained when ABMT was performed to treat the first relapse, with marrow collected at the beginning of the first remission according to the technical criteria outlined above.

In all but one case (treated with TACC) CR developed parallel to the kinetics of recovery of hematopoiesis which are typical of autologous engraftment, suggesting a link between the two events. Complete disappearance of the blast cells was seen in 30 of 33 cases and all deaths occurred in aplasia due to infectious, cardiac or respiratory complications. Thus these early results have shown the feasibility of the technique, the sensitivity of the blast cells to high-dose cytotoxic therapy and the possibility of achieving a higher remission rate than that obtained with conventional chemotherapy. In the absence of maintenance therapy the duration of remission obtained with TACC + autograft appears to be very similar to that of the remission during which the marrow was collected. If maintenance therapy follows ABMT, the remission duration and survival can surpass that of the previous remission. One of our patients with acute myelocytic leukemia (AML) diagnosed in May 1976 and treated with conventional chemotherapy for a first complete remission of 19 months was brought into a second remission lasting 4 yr by TACC + ABMT. At the second relapse, in May 1982, a third remission was achieved again by TACC + ABMT and persists at the time of writing. A few autografts have even been carried out during the first remission in order to reduce the residual tumor burden and hopefully to prolong this remission to cure.

We are aware of 10 AML patients treated in this way: 2 died in aplasia, 3 relapsed 3–9 months after autograft and 5 remain in unmaintained complete remission 7+ to 33+ months (7+, 18+, 24+, 24+, 33+) post-transplant. The results seem to be less encouraging for ALL.

The development of *in vitro* treatment of collected marrow to eliminate residual leukemic cells adds a new dimension to autografting. An active catabolite of cyclophosphamide, 4 hydroperoxycyclophosphamide (4 HC), destroys leukemic CFU, BFU-c and CFU-c *in vitro* [35] but spares murine CFUs [36]. In the rat model of AML it is possible to purge the marrow of leukemic cells which contaminate it: a healthy marrow inoculated with 10^6 myeloblasts (a dose higher than the LD_{100}) but treated *in vitro* by this derivative (at a dose of 60 nmol/ml) can reconstitute normal hematopoiesis after TBI without the ultimate development of leukemia [37]. In man ongoing trials show that the marrow treated *in vitro*, even with high doses of 4 HC and thus depleted of CFU-c, can retain its stem cell potential and be used for a successful autograft. Relapses have occurred in the first patients whose marrow was incubated with low doses of 4 HC. There is still insufficient information to comment on the effectiveness of high-dose 4 HC.

Polyclonal anti-leukemia antibodies have been used in a few patients [38] and are rapidly being replaced by monoclonal antibodies. The first trials with monoclonal antibodies were done in CALLA + ALL of children with the anti-CALLA antibody J5. Several *in vitro* incubations in the presence of the rabbit complement resulted in a 10^4 decrease in the level of tumor cells. Sixteen patients in second or third remission aged 2–20 have undergone transplantation after treatment

of their autologous bone marrow with the J5 and or J2 monoclonal antibodies. Six patients remain in complete clinical (CCR) remission with a median follow up of 14 months. Two patients are in CCR at 28+ and 30+ months, two at 13+ and 14+ months and two at 3 months +. There have been 10 failures: 5 leukemic recurrences and 5 remission deaths. The graft kinetics were satisfactory except for a few delays in the recovery of thrombopoiesis [28, 39].

Monoclonal antibodies against different types of leukemia are now available and are already being used actively for *in vitro* treatment. An additional *in vitro* antileukemic benefit is obtained with immunotoxins (IT), which are monoclonal antibodies coupled by a disulfide bond to the A chain of the ricin molecule. The antibody binds specifically to the blast cell and allows the A chain to enter it, leading to its destruction with a greater effectiveness than the monoclonal antibodies alone. The cytotoxic effect of the complement is not required and no immunomodulation has yet been described. *In vitro* tests evaluating cytotoxicity, as well as cures obtained by ABMT with marrow incubated with IT in the mouse B lymphoblastic leukemia model, indicate that there are very few, if any, residual tumor cells left [40, 41].

Numerous other techniques for treating the marrow *in vitro* are under study and some appear promising, especially lysozomotropic agents such as *N*-dodecylmorpholine, which is specifically toxic for blast cells and yet spares CFU-c, BFU-e and stem cells [35].

Based on the evolution in the last decade of the therapeutic trials for allogeneic bone marrow transplantation, it is predictable that ABMT with previously treated *in vitro* marrow will be used earlier in the course of the first remission. The absence of graft-vs-host reaction and the fact that two-thirds of patients have no compatible donor for allogeneic transplantation argue strongly in favor of this direction.

CHRONIC MYELOGENOUS LEUKEMIA

Chronic myelogenous leukemia (CML) is characterized by a chronic phase of a median duration of 36 months, an accelerated phase and a terminal phase of blast crisis.

In this last phase conventional chemotherapy succeeds in reverting the patient back to a chronic phase in only 30% of the cases, and the median survival is less than 6 months [42].

Theoretically the reinjection of autologous stem cells collected during the chronic phase ought to ensure a return to a second chronic phase of a similar duration to the first. To date, however, therapeutic trials have shown that there are difficulties with this approach. Goldman *et al.* have treated 33 CML patients in acute transformation with high-dose chemotherapy, TBI and reinfusion of the stem cells collected from the chronic phase peripheral blood. Thirty-two patients (95%) reverted to the chronic phase. No maintenance therapy was used. The reversion lasted a median of 13 weeks and the median survival was 23 weeks. Other investigators have obtained similar results with cryopreserved peripheral blood stem cells [43]. These studies show that, although a reversion to the chronic phase is easy to obtain, its duration is short, suggesting that either the blast clone responsible for the transformation is resistant to the treatment used or the peripheral blood stem cells are defective and have no selective advantage over the blast population: there has been very little experience with marrow stem cells in this disease and their effectiveness remains to be seen.

To our knowledge, the disappearance of the Philadelphia chromosome after autologous bone marrow transplantation has been reported in three instances [43–45]. In one case belonging to our own series the cryopreserved marrow was a genetic mosaic with 50% Philadelphia chromosome-negative cells. It appears that only those cells engrafted, to the detriment of the Philadelphia chromosome-positive population.

Although the results of ABMT in CML remain disappointing in terms of survival, the important fact that 95% of the cases can be reverted to the chronic phase must be emphasized. Reasonable hope for improvement could come from two major lines of research:

(1) The results over the last 10 yr show that allogeneic transplantation in CML is effective only when undertaken in the chronic or occasionally in the accelerated phase of the disease. On the other hand, when it is carried out during the acute transformation long-term survival occurs in less than 5% of patients due to the blast cell resistance to chemotherapy and/or radiotherapy. Based on this experience, it seems logical to perform ABMT earlier in the course of CML. The timing could be determined by the earliest appearance of a transformation clone detected by karyotypic examinations performed at regular intervals throughout the disease. One of our patients, grafted after the first detection of an abnormal clone (duplication of the Philadelphia chromosome and a supernumerary chromosome 8), when the percentage of marrow blasts remained unchanged, is still in chronic phase with a disappearance of the new clone on repeated karyotypes 14+ months later, at the time of writing.

(2) Performance of the autologous transplantation with only those stem cells which are Phi chromosome-negative would be an important step forward. Research in this direction has only just begun. One could collect the marrow after high dose chemotherapy, but this eliminates the Philadelphia chromosome-positive cells in only 50% of the cases [46]. An alternative approach would be to treat the stem cells *in vitro* with cytotoxic drugs. Also, there might be a possibility of destroying selectively the Phl chromosome-bearing cells by programmes of suboptimal freezing or even hyperthermia.

Very recently, it has been shown possible to select hematopoietic Philadelphia-negative progenitors (CFUc, BFUe and CFUGEMM) from the adherent layer of long-term Dexter type cultures initiated from marrow of patients in the chronic phase of CML bearing the Philadelphia chromosome [47].

NON-HODGKIN'S LYMPHOMAS (NHL)

It is in the treatment of NHL that the usefulness of ABMT is most firmly established. In preclinical studies using a dog model, Bowles and others [48] developed the first guidelines: 3 groups of about 10 dogs each, afflicted with spontaneous lymphomas, received total irradiation followed by an autograft. Group 1 received the total irradiation as an accessory consolidating treatment after having obtained a complete remission from conventional chemotherapy. The bone marrow used in the autograft had been collected after the remission took place. Group 2 had the same treatment except that the bone marrow, collected during remission, was purposedly contaminated with lymphomatous cells collected before the chemotherapy. In group 3, the total irradiation and autograft were done after relapse with raised tumor masses. The average survival in groups 1, 2 and 3 was respectively 216, 60 and 40 days, which suggests that the bone marrow ought preferably to be collected during a complete remission and that the combination of total irradiation and autograft give best results when they are used as accessory treatment for consolidation in cases with imperceptible tumor masses.

In man, the main therapeutic trials have been carried out in patients with NHL in relapse and resistant to conventional chemotherapy. In 27 patients, suffering for the most part from 'diffuse histiocytic' or 'diffuse poorly differentiated' lymphomas, after the classification of Rappaport, treated during a chemoresistant relapse by total irradiation (in a single dose or in fractionated doses), Philipps *et al.* [49] report a complete remission rate of 56% and a plateau of disease free survival from 20 to 60 months + in 26%.

In a total of 26 patients with NHL of poor prognosis, for the most part in progressive relapse, some in a second complete remission, Philip, Biron, Hervé, Maraninchi and others report a survival rate without detectable tumor of 58%. The treatment in this group was heterogenous, some patients receiving total irradiation, others polychemotherapy, BACT or TACC, and still others a combination of total irradiation and TACC [50]. Other authors have reported similar results in smaller series whether an autograft or a syngenetic allograft was used [51, 52]. Analysis of the results show that in the highly malignant group of NHL, the lymphomas of Burkitt are the most sensitive to heavy chemotherapy, BACT or TACC + ABMT [53].

On the whole, it has now been established that use of ABMT as a salvage therapy results in a cure in a non-negligible percentage of patients suffering from NHL in active relapse beyond help from conventional chemotherapy.

The use of ABMT during the first complete remission in cases with a poor prognosis, in order to allow a treatment of consolidation with heavy chemotherapy seems reasonable. In a preliminary trial [19], 12 patients with NHL of poor prognosis were treated by TACC and ABMT and divided into 2 groups depending on whether the treatment was for a large mass of tumor tissue (group 1: 3 recently diagnosed and 3 relapsed cases) or for consolidation of remission in cases with undetectable tumor (group 2: 6 patients). In the first group 3 complete remissions (8, 24+, 48+ months) were obtained in the 3 recently diagnosed cases and 3 failures in the relapsed cases. In the second group there were 5 complete remissions of long duration (15+ to 40+ months). In addition, none of the cases in group 2 suffered a septicemia, while in group 1 sepsis occurred in 5 of the 6 patients. Important recent advances in chemotherapy for inducing remissions, giving a remission rate around 70% persisting up to 2 yr, call for randomized trials in order to determine the value of a consolidation treatment associated with ABMT during a first remission. A cooperative trial of this type is currently underway in NHL of poor prognosis to compare conventional chemotherapy (M-BACOD) used alone with the same chemotherapy followed by consolidation with high-dose combination therapy + ABMT.

As in acute leukemia, recent developments give rise to hope that a new approach has been found for treating lymphomas with medullary invasion. In the case of B lymphoma, notably, attempts to eliminate the invading cells from the bone marrow with murine anti Y 29/55 monoclonal

antibodies seems to have been successful. Four patients in a first remission consolidated by chemotherapy, total body irradiation and ABMT with marrow thus treated remain up to now in complete remission without maintenance therapy for 3–20 months [54].

On the whole, ABMT has now an established place as a second line therapy of NHL. On the other hand, its place in primary treatment and in cases with medullary infiltration is still under study.

SOLID TUMORS

It is difficult to speak of an overall role of ABMT with respect to solid tumors because of their wide heterogeneity. It is necessary to consider each type of tumor based on its own characteristics, most important of which are its chemosensitivity and its propensity to metastasize to the bone marrow. Moreover, some use ABMT as a support for phase II trials, where the antitumor effectiveness as well as the extramedullary side-effects of the drugs are evaluated. Others already integrate ABMT in phase III trials in which high-dose chemotherapy + autotransplantation take their place directly in the antitumor strategy. As an example of phase II trials, a study of high-dose melphalan for metastic malignant melanoma [55, 56] has shown that doses up to 180 mg/m^2 could be used, resulting in a 63% response rate with 18% complete remissions; however, the 1-yr disease-free survival was only 10%.

There are many phase III trials going on for the oat carcinoma of the lung. Symann and others [57] have carried out a randomized trial in patients with this cancer, put into remission by conventional chemotherapy, combining vincristine, cyclophosphamide, doxorubine and methotrexate. One half of the patients received a strong additional treatment of cyclophosphamide (6 mg/m^2), VP 16 (500 mg/m^2) and BCNU (300 mg/m^2) followed by ABMT, while the other half received only an additional course of the induction regimen. Of the 77 original patients, 30 reached a stage suitable for randomized study. Thirteen (6 localized and 7 disseminated cancers) were treated by ABMT with the following results: the 6 patients with localized cancer who were autografted have survived with no discernable tumor for 2+ to 26+ months. By contrast, only 4 patients out of 12 not autografted remain in remission over the same period of time.

In the disseminated forms, 2 out of the 7 patients have survived free of tumor (with a short follow up of 3 months) and 1 patient out of 5 of the control group has survived, but in a state of relapse.

This study shows the value of using a heavy consolidating treatment associated with ABMT in oat cell carcinoma of the lung, but indications are reduced to tumors initially localized and previously put in complete remission by conventional chemotherapy.

Other therapeutic trials are being carried out to determine the value of intensified treatment associated with ABMT in patients with adenocarcinoma of the ovary, testicular choriocarcinoma and glioblastoma, and still more recently in young women with highly inflammed breast cancer in whom attempts to fractionate the collected bone marrow to purge it of possible cancer cells are in the course of being assessed [58].

In any case, it seems reasonable to propose heavy chemotherapy with autografting to consolidate results in patients who are in complete or partial remission from conventional chemotherapy, which is to say, in favorable cases with small residual tumor and high sensitivity to heavy chemotherapy [59].

CONCLUSION

It is difficult today to predict the future of autologous bone marrow transplantation. In oncology the ongoing trials will clarify its role, and for those tumors which are not very chemosensitive ABMT may offer a last chance to chemotherapy. In hematology its use in the treatment of high- and intermediate-grade NHL is being confirmed rapidly. We believe that it is in the treatment of acute leukemia that ABMT is the most promising: one can imagine that in the coming decade the techniques to purge the marrow collected in remission of residual leukemic cells will be reliable. ABMT may then become an indispensable step in treating leukemia and may compete with allogeneic bone marrow transplantation, especially if the latter becomes feasible from unrelated donors. It is very likely indeed that both techniques will coexist or disappear together if the long-awaited etiopathogenic therapy of acute leukemia is at last discovered.

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