Reviews in Medicine

Haematology

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Introduction

The commission to review the advances in haematology for one year forces one to consider the size of the task, in terms of papers published, it is enormous; for instance in 1989, the journal 'Blood' had 16 issues comprising over 5,000 pages and a total of some 3 million text words. This output by one journal reflects the overall growth in medical publishing since the second world war (see Figure 1). As it is not plausible that authors will restrict their outpourings, any limit on this ludicrous quantity of print must be imposed by an outside body. In the first instance I favour the adoption of three proposals:

- no applicant for a job should cite more than 6 publications (10 for full professors)
- control of multiauthor papers and gift authorship by limiting the number of authors cited in *Index Medicus* to the first six
- no *Index Medicus* citation for any article whose cost has been defrayed by the author paying page charges (e.g. \$50 per page for *Blood*).

This hobby horse dealt with, I shall attempt to highlight papers published over the last year which reflect growth areas in haematology and which may be of interest to practising haematologists and physicians with an interest in haematology. My selection is personal; I have also attempted to put growth areas into perspective and to appraise critically some of the published work.

Haemopoietic growth factors

There has been increasing interest in growth hormones (colony stimulating factors, or cytokines) which regulate the proliferation of bone marrow progenitor cells and control the function of mature blood cells. Molecular biology has allowed the production of large quantities of recombinant factors, which are now being introduced into clinical practice.¹ It is becoming clear that the

various cytokines act within a complex network of inter-reactions, thus, isolating the effects of one particular growth factor is difficult, and possibly futile since each cytokine operates within an environment produced by other growth factors. These cytokines exert their effect on target cells by binding to high affinity cell surface receptors, which are present in relatively low concentration on haemopoietic cells.

(a) Erythropoietin

Erythropoietin is a glycosylated protein with a molecular weight of 34,000: over 90% of the hormone is produced by the kidney and its action is lineage specific.^{2,3} In normal individuals this hormone is released in response to renal hypoxia and

Figure 1 Articles published and indexed in *Index Medicus* (in thousands) for the years 1948, 1958, 1968, 1978 and 1988 (data kindly provided by the National Library of Medicine, Bethesda, MD).

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stimulates erythroid precursors in the marrow to increase red cell production, providing a simple control mechanism. Severe hypoxia is the stimulus for erythropoietin production and indeed the hormone was first isolated from the urine of patients with aplastic anaemia.⁴

Patients with end-stage renal failure are usually anaemic and may require regular transfusion.² This anaemia is principally due to an impaired marrow response because of erythropoietin deficiency,² and early trials demonstrated the effectiveness of recombinant erythropoietin therapy in reversing such an anaemia,^{5,6} thus making the patient transfusion-independent and avoiding the potential risks of regular blood transfusion: transmitted viral infection and iron overload.^{7,8} Erythropoietin dosage should be increased slowly to try and prevent the development or worsening of hypertension.⁹ Delay in response to treatment implies another cause for anaemia, particularly an underlying iron deficiency.^{7,9,10} Recently, two reports on over 450 patients have proven the efficacy and safety of this treatment,^{7,9} with patients having a measurable increased exercise capacity and improved cardiac and respiratory function.¹¹ There is however an increased risk of thrombosis of fistulae,^{7,9} and a slight increase in other thrombotic events (stroke, myocardial infarction). Erythropoietin therapy is therefore of proven value but it is expensive: at current costs around £5,000 per patient per year.⁸ Obviously treatment is indicated in transfusion-dependent individuals but it seems likely that some form of rationing may need to be introduced for other patients, and there is debate about the criteria which should be used to evaluate a particular patient's needs. A related issue is the target haemoglobin level (since a high haemoglobin increases the risk of thrombotic sequelae and is more expensive to achieve). It has been suggested that a haemoglobin of 95–115 g/l may give most benefit without risk.⁸ It is of note that the haemostatic defect seen in renal failure¹² is reversed by normalization of the haemoglobin with erythropoietin therapy.¹³ Erythropoietin is also successful in treating the anaemia seen in human immunodeficiency virus (HIV) positive patients on zidovudine therapy¹ and in rheumatoid arthritis, although these latter patients do not benefit symptomatically.¹⁴

(b) Granulocyte-macrophage colony stimulating factor (GM-CSF)

T-lymphocytes and monocytes both produce GM-CSF in response to inflammatory stimuli. GM-CSF is produced locally and is not detectable in the serum. GM-CSF acts locally as a multipotential haemopoietic growth factor stimulating production of granulocytes and macrophages.¹ GM-CSF

also regulates the function of neutrophils, eosinophils and macrophages as well as inhibiting neutrophil migration, thus acting to localize an immune response.

In clinical trials GM-CSF has been used to correct the chronic severe neutropenia seen in patients with both congenital and chronic severe neutropenia.¹⁵ GM-CSF shortens the neutrophil recovery time after chemotherapy-induced neutropenia,^{1,16} and its major application is likely to be in speeding up marrow recovery following chemotherapy or bone marrow transplantation (BMT). This will thus reduce the number of days' antibiotic therapy that patients require but is unlikely to have much impact on mortality since relatively few patients die between day 10 and 21 post-BMT. GM-CSF treatment is generally well tolerated although there may be some bone pain, gastrointestinal disturbances and itching.¹⁷

GM-CSF may be of most value in patients who would currently require autologous BMT. Thus in patients with non-haematological malignancy GM-CSF may permit the use of very aggressive chemotherapy regimens without the need for autologous BMT.¹⁸ Alternatively GM-CSF can be used to improve the harvest in patients undergoing autologous BMT: cyclophosphamide is given to induce pancytopenia and is followed by GM-CSF which produces a dramatic increase in the number of circulating stem cells. These cells can be harvested by leucapheresis and have been successfully used to achieve a rapid haematological recovery after lethal chemoradiotherapy.¹⁹ These approaches are likely to increase the role of very high dose chemotherapy in the management of both haematological and non-haematological malignancies.

Trials of GM-CSF in patients with aplastic anaemia have shown an increase in the circulating neutrophil numbers (1.5- to 20-fold) but there is no appreciable effect on the platelet count.¹⁵ However, the effect is short-lived and GM-CSF may act principally to mobilize marginated neutrophils, since there was no observable increase in the myeloid colony forming units. Clearly GM-CSF cannot be expected to exert a major effect if the marrow pluripotent stem cells are not healthy, and as withdrawal of the drug results in relapse,¹⁵ it would seem that GM-CSF, given alone, has little to offer in aplastic anaemia. Similarly, in the treatment of myelodysplasia GM-CSF has increased the neutrophil count. Unfortunately, as with aplastic anaemia, this effect is only observed when the patient is receiving treatment and there is no appreciable effect on other cell lineages.^{1,20} Lastly, in those patients with myelodysplasia and an excess of myeloblasts before starting GM-CSF there appears to be a considerable risk of precipitating the development of acute leukaemia.²¹

Up to 25% of patients may develop antibodies to

yeast-derived GM-CSF, because of the presence of unprotected glycosylation sites.²² The clinical significance of this retrospective finding is unclear but it may limit the use of growth factors derived from yeast, and perhaps other non-mammalian systems.

(c) Granulocyte colony stimulating factor (G-CSF)

G-CSF is a relatively lineage-specific growth factor that supports the differentiation of marrow progenitor cells which are committed to the neutrophil lineage.¹ G-CSF has been successfully used to reverse idiopathic neutropenia,²³ congenital agranulocytosis,²⁴ drug-induced agranulocytosis²⁵ and to induce remission in hypoplastic acute myeloblastic leukaemia.²⁶ G-CSF has also been used following cytotoxic therapy and autologous BMT to reduce the period of neutropenia and thereby significantly reduce the in-patient stay required.²⁷ However, as with GM-CSF this is unlikely to make much difference to procedure-related deaths. G-CSF therapy has been shown to enable the delivery of more chemotherapy to patients, compared with controls in whom myelosuppression may delay the next cycle of therapy.²⁸ Its major benefit may therefore be in permitting the delivery of more chemotherapy to patients with malignant disease.

More experimentally, G-CSF has been tried in patients with myelodysplastic syndromes and produced an impressive increase in neutrophil count, with no apparent risk of enhancing leukaemic transformation.²⁹

(d) Other cytokines

There has been less published work on other growth factors although interleukin 3 appears to be particularly promising. This is a multipotential growth factor which appears more potent than GM-CSF at stimulating early marrow progenitors.¹ Clinical studies with this agent are currently in progress.

(e) Inhibitors of haemopoietic stem cell proliferation

Proteins which inhibit the proliferation of pluripotent stem cells have recently been identified.^{30,31} These proteins are produced by macrophages and may act to switch off cell growth stimulation and could be used to keep haemopoietic stem cells from entering the cell cycle during the administration of chemotherapy, thereby protecting them from the effects of cytotoxic drugs. Since marrow suppression is usually the dose limiting factor in chemotherapy regimens it can be seen that such agents would have the potential to improve the treatment of chemotherapy-sensitive neoplasms.³¹

Aetiology of acute leukaemia

Patients and their physicians are always keen to understand why particular malignant processes occur. However, leukaemia is an uncommon event so that the effect of any postulated aetiological agent will only be recognized as an increased risk compared with the normal population, i.e. an increased 'relative risk'. It is important to realize that the numbers of cases involved in a 'cluster' may be very small, and thus prone to random variation which should not be overinterpreted. Indeed, publication of small studies may lead to considerable media attention and induce undue anxiety, as has recently been observed in relation to the television programme on 'leukaemia and power lines'.³²

(a) Radiation

One acknowledged aetiological factor for leukaemia is radiation: evidence for this association comes from the Japanese survivors of atomic bombs, individuals irradiated *in utero*, and patients given high dose radiotherapy for ankylosing spondylitis.³³ In contrast, diagnostic X-rays and radiotherapy for Hodgkin's disease have not been linked to leukaemia, so it can be seen that a dosage effect is apparent.

Controversy particularly surrounds the apparent clustering of childhood acute lymphoblastic leukaemia (ALL) observed around some nuclear plants: particularly the reprocessing plant at Sellafield in West Cumbria.³⁴⁻³⁶ Although the use of postal districts to detect such an effect is open to over zealous interpretation, sophisticated analysis with a Geographical Analysis Machine confirms that there is a high incidence of childhood ALL around Sellafield.³⁷ In contrast, no leukaemia hot-spot appears to exist around nuclear reprocessing plants at Dounreay³⁸ or Le Hague³⁹ whilst there is an even bigger cluster in Tyneside which is more than 50 km away from the nuclear industry.³⁷ It has already been demonstrated that there is no significant risk of leukaemia or other cancers in radiation workers from Sellafield⁴⁰ or other atomic energy sites.⁴¹ It is also of note that the incidence of Hodgkin's and non-Hodgkin's lymphoma was not increased.

Gardner believed that radiation may be responsible for some cases of childhood ALL and therefore pursued the geographical link established at Sellafield, studying numerous parameters in ALL patients compared with 'case-controls'.^{35,36} Over a 35-year period there were 52 cases aged less than 25 within the West Cumbria health district. Nine of these were children born to men working at the nuclear plant (17.3%) compared with case controls in whom only 41/277 (14.8%) had fathers working at the plant. A close worked statistical

analysis gave rise to the hypothesis of a link between father's occupational exposure to radiation prior to conception (especially radiation dosage above 100 mSv) and leukaemia developing in the offspring (the relative risk being quoted as up to seven times that of a control population).^{35,36}

There is also limited experimental evidence to suggest that such a mechanism may occur.⁴² However, three caveats should be sounded: (i) this hypothesis is actually based on only 5 cases of ALL, (ii) there is also a risk of a first degree statistical error, given the number of possible associations studied and (iii) a large study of children born to the 7387 male survivors of the Hiroshima and Nagasaki bombs did not show an increased risk of leukaemia.⁴³ In conclusion this theory must be proven at other sites before being accepted, but until this question is settled the reduction in maximum exposure levels from 50 mSv to 15 mSv should allay some of the anxiety in employees at Sellafield.⁴⁴

It seems likely this matter will remain unresolved for some time to come, and until then the claims for compensation against British Nuclear Fuels are premature. Even if causation were proved by tort, the award of huge sums to a tiny group of children or their families would be in sharp contrast to all other patients with this disease, seeming both illogical and unfair.

(b) Viruses

There is increasing evidence that viral infection may be an important step in the causation of some lymphoid malignancies. Thus adult T-cell leukaemia/lymphoma (ATL) is only observed in those previously infected with HTLV I (human T-cell leukaemia/lymphoma virus); HIV infection predisposes to high grade B-cell lymphomas and Hodgkin's disease and Epstein-Barr virus is intimately concerned in the development of Burkitt's lymphoma and possibly Hodgkin's disease. Therefore, in some instances viral infections are a prerequisite for later leukaemogenesis. Viral infection may be central to the development of childhood ALL, even if leukaemia only occurs in a very small proportion of those exposed to the virus, and the timing of viral infection may be crucial to the risk of a later leukaemia. Thus an alternative explanation for the ALL cluster at Sellafield is based on the finding that clusters have also been observed around places considered for nuclear reprocessing plants but where no such plant was built.⁴⁵ Like Sellafield such sites are geographically remote and sustained a large population influx in the 1950s. Kinlen has suggested that people in this area had a low herd immunity to common viral infections (as in measles) and that a rare response to one of these is

the development of ALL.⁴⁶ This might also explain the apparent cluster of childhood ALL seen in my home county, Shropshire.⁴⁷

(c) Drugs and chemicals

Cytotoxic therapy, particularly with alkylating agents is known to increase the risk of later acute myeloblastic leukaemia (AML).⁴⁸ This is particularly recognized in patients who receive combination chemotherapy for Hodgkin's disease: patients have a cumulative rate of developing myelodysplasia or leukaemia of around 10% by 10 years after completion of therapy.^{49,50} There is a temporal relationship to this risk in that it first becomes apparent one year after starting chemotherapy and has disappeared a decade after stopping these drugs.⁴⁹ The risk of alkylating agents (chlorambucil, melphalan, cyclophosphamide) was again emphasized by a recent paper describing a 12-fold increased risk of leukaemia in patients who survived ovarian carcinoma after being treated with these agents.⁵¹ Similarly, exposure to benzene has long been recognized as a potential cause of aplastic anaemia and a risk factor for AML.^{52,53} This gained international prominence in the lay press when it was found that some carbonated mineral waters contained benzene and were withdrawn from supermarket shelves, even though the levels were not outside recommended exposure limits.

Leukaemias occurring after chemotherapy are notoriously difficult to treat and it has now been shown that chemotherapy or benzene-associated leukaemias are frequently accompanied by non-random chromosomal changes: especially loss of part of the long arm of chromosome 5 or 7, or complete loss of either chromosome.⁵⁴ This combination of (i) leukaemia associated with defects of chromosome 5 or 7, (ii) prior exposure to a carcinogen and (iii) a consistent time lag, implies causality.^{48,54,55} Furthermore, it is tantalizing to discover that the critical region on the long arm of chromosome 5 contains the gene for numerous growth factors (granulocyte-macrophage CSF, interleukin 3, interleukin 5 and platelet-derived growth factor),⁵⁵ whilst the gene encoding erythropoietin is situated at 7q22. It seems that the deletion of these or related genes is central to the pathophysiology of these therapy related leukaemias.⁴⁸

Indeed, cytogenetic abnormalities are being increasingly recognized as having prognostic importance in patients with AML. Thus studies have confirmed that cytogenetic abnormalities involving 5 or 7 are associated with a poor prognosis.^{56,57} Two large studies have also confirmed the associations between various non-random cytogenetic abnormalities and FAB subtypes of AML: t(8;21) with M2 AML, t(15;17) with M3 AML,

inversion 16 with M4 AML and eosinophilia. This latter leukaemia is usually found in younger patients and has a relatively favourable outlook.^{56,57} More recently, deletions from the short arm of chromosome 9 have been described in patients with acute lymphoblastic leukaemia.⁵⁸ This deletion site is close to the genes coding for interferon and it has been suggested that these deletions may lead to the loss of a tumour suppressor gene, possibly even the interferon gene.⁵⁸

Treatment of acute leukaemia

There continue to be reports of large scale trials which increasingly show improved results,^{59,60} and are usually based at many centres. This advance has relied on the use of aggressive combination chemotherapy, which leads to prolonged pancytopenia requiring considerable support for infective and bleeding episodes. It should be emphasized that much of the improvement is attributable to the provision of better supportive care during chemotherapy-induced pancytopenia.⁶¹ However, the chemotherapy is clearly of primary importance and in a review of over 4000 children with acute lymphoblastic leukaemia it was clearly shown that patients entered into trials had a better long term survival than those not in trials.⁶⁰ Equally important, it was shown that patients treated in small centres fared as well as those in large centres provided that they were entered into trials.⁶⁰

The treatment of acute leukaemia can be divided into 3 phases: supportive care, induction of remission and further therapy.⁶² Clearly the outlook for patients who fail to enter remission is bleak, and most studies concentrate on the best way to treat minimal residual disease. The 'best option', chemotherapy or bone marrow transplantation (BMT), for a particular patient, depends on many factors: age, underlying disease, disease status, presence of adverse prognostic factors and donor availability.^{63,64} Confusion over the best choice is confounded by protagonists of one therapeutic option publishing over-optimistic results based on a small series of highly selected patients and providing embarrassingly short follow-up. In many papers it is difficult to find the time of minimum follow-up and this deficiency is obscured by statistical methods which allow the authors to 'extend' the follow-up and quote an actuarial relapse risk at some future date. In 1987 a *Lancet* editorial advocated that an acceptable format for communications on bone marrow transplantation should include a minimum 2 year follow-up on every patient⁶⁵ but this is usually woefully lacking. Large ongoing studies should help to answer the question of which patients should be offered BMT, and when. However, most of the trials are not

considering the impact which haemopoietic growth factors (*vide supra*) or interleukin 2 therapy⁶⁶ may give.

(a) Chemotherapy

The long term efficacy of chemotherapy in patients with acute myeloblastic leukaemia (AML) is only now becoming apparent, since previous studies have reported on cohorts with a relatively short follow-up. The CALGB group has now published prolonged follow-up on 760 patients who entered remission of AML;⁶⁷ these cases were on maintenance treatment. Unfortunately median remission time was only 13 months, and 3 years post-remission only 25% patients remain disease-free. Furthermore these data suggest a continual relapse rate up to 8 years post-remission and, depressingly, there was no identifiable disease-free plateau which would be expected if patients were being cured of AML by chemotherapy alone.⁶⁷ These results were confirmed in a Danish study of patients who were in first remission 3 years from obtaining this remission.⁶⁸ However, this study is more optimistic in showing that the relapse rate after 3 years is relatively slow, so that 60% of these patients will appear 'disease free' 5 years later (i.e. 8 years after diagnosis).

(b) Bone marrow transplantation (BMT)

BMT may be carried out using a matched sibling donor (allogeneic) or the patient's own marrow may be reinfused (autologous). Autologous BMT offers the possibility of myeloablative chemo/radiotherapy without the risks of graft-versus-host disease (GvHD).^{65,69,70} However, in patients with marrow involvement such an approach will lead to the reinfusion of malignant cells and, this has led to the introduction of purging protocols, although these are of unproven value.⁶⁵ Lately, a combination of ether lipids and hyperthermia to 42°C for one hour has been shown to inhibit leukaemic progenitor cell growth to less than 3%.⁷¹ An alternative strategy relies on the premise that, following cytotoxic therapy, normal, rather than leukaemic, stem cells are released into the circulation. Thus patients may be given cytotoxic therapy to induce pancytopenia and circulating stem cells are then harvested by leucapheresis: this approach has yielded variable results.

In patients who undergo allogeneic BMT there is increasing evidence that GvHD and its prevention are crucial elements in obtaining a cure of the underlying disease. Although GvHD may be associated with life-threatening complications it appears to be important as 'immunotherapy' for acute leukaemia.⁷² Thus prophylaxis against GvHD may influence the leukaemic relapse rate:

the most commonly used techniques involve regular methotrexate therapy, T-cell depletion or cyclosporin. Results from over 600 patients have shown that methotrexate reduces the risk of leukaemic relapse in comparison with cyclosporin or T-cell depletion of the donor marrow,⁷³ and the evidence suggests that this is due to an anti-leukaemic effect of methotrexate rather than less effective suppression of GvHD.⁷³

One group has attempted to harness the graft-versus-leukaemia effect of GvH by inducing 'GvHD' in patients undergoing autologous BMT.⁷⁴ Although this appears to ignore the definition of GvHD,⁷⁵ the authors claim to have stimulated host lymphocytes to be cytotoxic against self. Clearly this work needs further clarification.

Comparisons between allogeneic and autologous BMT for patients aged less than 50, in first remission of AML, are being carried out in many multicentre trials. Early data from small studies suggests that allogeneic BMT gives a higher probability of prolonged disease free survival,^{76,77} although studies on such small patient numbers must be regarded with some caution. Although allogeneic BMT is proven to offer a good chance of long term cure, this has not yet been demonstrated for autologous BMT; indeed there is no convincing evidence that survival curves reach a plateau phase after autologous BMT.⁷⁰ It is quite possible that autologous BMT, for AML, is no more effective at achieving a cure, than 'high dose' consolidation therapy and is merely prolonging the duration of remission before late relapse. Thus autologous BMT should only be carried out within the context of a scientific study, so that its role in acute leukaemia can be properly assessed.^{65,70}

Allogeneic BMT is only feasible in patients aged less than 50 who have an HLA-identical sibling. Only 35% of patients will have a matched sibling to act as a potential marrow donor, so there has been an increasing interest in the use of matched unrelated donor (MUD) BMT.⁷⁸ This procedure is very experimental and carries a higher risk of GvHD and hence death than matched sibling BMT. My experience with 14 patients subjected to MUD BMT has been bad: only one patient survived longer than one year. These patients are at risk of developing savage fatal GvHD ('the blazing spitfire syndrome', a term coined by Francis Matthey). Therefore the decision to perform a MUD BMT in a patient with AML in first remission, or in first chronic phase of CML is contentious. I believe this experimental approach should be restricted to only one UK transplant centre, so that expertise can be concentrated in this unit and the value of MUD BMT be properly evaluated.

Aggressive therapy for multiple myeloma

The treatment of this depressing condition has shown little improvement over the last several years and pulsed doses of melphalan and steroids remain the mainstay of treatment.⁷⁹ In most patients the paraprotein level falls until a plateau is reached; therapy should then be discontinued. Unfortunately, only two thirds of patients respond and even in these individuals disease progression eventually occurs, so that death is inevitable. Thus the median survival of patients diagnosed as having myeloma is only 3 years. Comparing different treatment protocols is difficult because of the heterogeneity of this disease, poor markers for prognosis (or staging), and because there has been no accepted criteria by which to judge disease response. Lately two groups have suggested broadly similar criteria for the definition of 'complete remission' which are: (i) no paraprotein in blood or urine, (ii) less than 5% plasma cells in the marrow and (iii) that this state should persist for 3 months.^{80,81} The universal adoption of this or of a similar definition would certainly simplify comparisons between different studies. Unfortunately the problem of a useful and acceptable staging system remains. Recent papers have suggested two ways in which the bleak outlook of myeloma may be improved.

(a) Intensive therapy from diagnosis

In younger patients pulsed courses of cytotoxic drugs may be used to reduce dramatically the tumour burden. The regimens used rely on a combination of vincristine, adriamycin and high dose steroids (similar to the VAD regimen piloted by Barlogie.⁸²). Such an approach is only appropriate in individuals who are able to withstand such treatment, and should probably be restricted to those aged less than 60. The major risk of such therapy is infection secondary to neutropenia, so prophylactic cotrimoxazole may be used. Up to 50% of patients may obtain a complete remission.^{80,81,83} and this may give a longer plateau phase before disease relapse.

(b) Maintenance of remission or plateau phase

Autologous bone marrow transplantation is being investigated as a means of consolidating complete remission, once achieved. This procedure is relatively well tolerated and early studies suggest that it may prolong disease-free survival.^{80,84,85}

An alternative is the use of interferon as 'maintenance' therapy in patients who reach plateau phase of their myeloma. One preliminary study suggested that patients who received alpha interferon had a longer survival than controls.⁸⁶

There are some worries about this work viz. (i) the number of patients was small and the disease course of myeloma is very variable⁷⁹ and (ii) the control group fared much worse than one would usually have expected. However, interferon may prove to be of benefit in myeloma and its role in the management of this disease is now being evaluated in large trials.

These approaches to treatment are experimental and controversial: indeed a recent Swedish study concluded that melphalan/prednisolone treatment is as useful as the more complex multidrug regimens.⁸⁷ This emphasizes the need to restrict new therapies to patients who are entered into large well organized multi-centre trials.

***In utero* bone marrow transplantation**

The feasibility of using liver haemopoietic cells to undertake bone marrow transplantation (BMT) in monkey fetuses within the womb has been demonstrated.⁸⁸ Transplants were carried out in 5 monkey fetuses at around 8 weeks' gestation, opposite sex donors being used so that karyotype analysis could be used to define successful engraftment. Such analysis showed successful engraftment in 4 of 5 monkeys with 5–15% of marrow precursors being of donor origin and persisting at this level for up to 2 years post-BMT.⁸⁸ This procedure has been proposed as potential therapy for patients with a congenital haemoglobinopathy *in utero*. This work is an impressive tour-de-force but I rate it as ill conceived as the Sinclair C5 (a tiny electric powered motorcar introduced in the UK in 1985, which redefined the concept of marketing failure). Firstly, the procedure would be expensive and divert resources from other needs but more worryingly it would enable the germ cells to be carried through to the next generation, in complete disregard of Darwinian natural selection.

Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) is a myeloproliferative disease with a typical cytogenetic abnormality: the Philadelphia chromosome. This fusion chromosome is formed by a translocation of the oncogene *c-abl* from chromosome 9 to the break point cluster region (*bcr*) on chromosome 22, t(9;22). This translocation gives rise to a *bcr/abl* P210 protein and it has recently been shown that this protein can induce a myeloproliferative state in mice: analogous to CML.⁸⁹ Failure to detect the Philadelphia chromosome, using standard cytogenetic methods may be caused by a much smaller transfer of material from chromosome 9 to 22; so that proof of the *bcr/abl* translocation relies on

molecular biological techniques.⁹⁰ An alternative explanation for Philadelphia negative CML is that the haematological diagnosis is wrong: thus review of a small series demonstrated that if no *bcr/abl* translocation was detectable, none of these cases fulfilled the criteria for the diagnosis of CML.⁹¹

The myeloproliferative state is typically characterized by splenomegaly, marked leucocytosis due to an increase in mature and immature myeloid precursors (usually in the range $50\text{--}400 \times 10^9/\text{l}$), thrombocytosis, moderate anaemia and a grossly hypercellular bone marrow. Treatment of this 'chronic phase' is straightforward and relies on pulsed doses of high dose busulphan or continuous hydroxyurea. Unfortunately chronic phase CML is an unstable state and acute leukaemia supervenes in nearly all patients. This blast transformation usually occurs within 3 years of diagnosis, although a few patients may remain in prolonged chronic phase.^{92,93} Painstaking efforts have been made to establish prognostic markers to predict the onset of blast crisis⁹⁴ but they are of limited value, since the change appears to be random. One of the most valuable prognostic criteria may be the duration of first remission as an indicator of the probable length of chronic phase (i.e. the time between achieving a white cell count of less than $10 \times 10^9/\text{l}$ and the need for more therapy).^{92,93} Four fifths of the acute leukaemias which arise in these patients are myeloid and resistant to therapy, with death usually occurring within a few weeks.

Considerable attempts have been made to improve the bleak outlook for CML but the only proven advance has been the introduction of allogeneic bone marrow transplantation (BMT). BMT offers the possibility of cure^{95,96} but is only possible in a minority of patients and it is worrying that many of these may undergo a cytogenetic relapse of their disease.^{95,96} The presence of the leukaemia specific *bcr/abl* translocation and the introduction of the polymerase chain reaction means that residual CML cells can be detected at a concentration of less than 1/10,000.⁹⁷ The incidence of cytogenetic relapse is unclear but one group has demonstrated a translocation in 11/12 patients who appeared to be in cytogenetic remission following BMT⁹⁸ and although the significance of a cytogenetic relapse is not yet proven it may predict disease relapse. Frank leukaemic relapse is particularly observed in patients who undergo a T-cell depleted transplant.⁹⁶

Matched sibling BMT is only feasible in 35% of patients under the age of 50, so efforts have been directed at other treatment modalities which may prolong the duration of chronic phase CML. Recently there has been considerable interest in the use of alpha interferon which controls the myeloproliferative state in 70% of cases, and early reports suggest it may delay the onset of blast cell

crisis,⁹⁹ with the Philadelphia chromosome becoming undetectable in a minority of cases. As 30% of patients do not respond to interferon, sceptics might suggest that response to alpha interferon merely identified those patients who belong to a relatively good prognostic category and who can therefore be expected to remain in remission for longer than non-responders. Other worries about the potential value of this treatment are that a significant number of patients develop antibodies to recombinant interferon,¹⁰⁰ and side effects may be severe enough for many other patients to discontinue therapy (*vide infra*). The results of large scale trials of this drug are awaited with interest.

Essential thrombocythaemia

Essential thrombocythaemia (ET) is a diagnosis of exclusion and can only be properly made if there is no underlying cause for a reactive thrombocytosis. Correct evaluation of a patient thus relies on excluding underlying carcinoma, iron deficiency, active blood loss, infection or any other inflammatory process. A bone marrow examination must also be carried out, in order to exclude the Philadelphia chromosome. However, even rigorous care will leave the diagnosis unclear in some patients; typically with a platelet count in the region $800-1200 \times 10^9/l$. A recent paper on the diagnosis of ET encourages the use of positive diagnostic criteria to differentiate this disorder from a reactive thrombocytosis:¹⁰¹ the scoring system is based upon five variables as shown in Table I.

Effective treatment of ET relies on adequate control of the platelet count, although there is no consensus on whether the level should be held at 800, 1000 or $1200 \times 10^9/l$. This can be readily achieved with pulsed doses of busulphan or continuous low dose hydroxyurea. These drugs do impart a very slight increase in the relative risk of developing leukaemia after several years, and this has led to trials of other therapies. Alpha interferon

has been shown successfully to control the thrombocytosis seen in ET and other myeloproliferative states.¹⁰²⁻¹⁰⁴ Although such therapy may control the thrombocytosis, the clinician should be well aware of the side effects which may be suffered by the patient; particularly psychological problems which may persist until the interferon has been discontinued. As ET is a disease which can be expected to run an indolent course and is easily controlled with chemotherapy, the issue of quality of patient life should rate very highly with the haematologist. Personally, I believe it is wrong to submit patients over the age of 60 to regular injections which may carry a constellation of side effects. This may be especially important since a proportion of patients will develop neutralizing antibodies to recombinant interferon, and therefore resistance to therapy.^{100,105} It should also be pointed out that the cost of treatment per patient per year may be around £2250.

Although the aetiology of ET is unclear, an interesting study claimed to have detected retrovirus within the platelets of 11 out of 11 patients with myeloproliferative disease with comparison to only 1 out of 7 control subjects.¹⁰⁶ The validity of such a finding needs to be confirmed since the techniques used may give rise to false positivity, but if this finding is confirmed it raises interesting questions as to the aetiology of these disorders.

Chronic lymphocytic leukaemia

The heterogeneity of this disease has been emphasized, including the importance of making an accurate diagnosis based on the observation of a sustained lymphocytosis (usually greater than $10 \times 10^9/l$): which is due to a clonal lymphoid proliferation, usually of B-cell origin. The International Workshop on chronic lymphocytic leukaemia (CLL) has recommended the adoption of the Binet staging system, rather than the older Rai system¹⁰⁷ (see Table II). This system is easier to

Table I Criteria for the diagnosis of essential thrombocythaemia and the scores allocated for each feature

Splenic enlargement on scan	2
Unstimulated BFU-E (*) derived clones from peripheral blood	2
Elevated platelet nucleotide ratio (ATP:ADP > 3.25)	1
Elevated platelet distribution width (PDW > 17)	1
Clinical ischaemia present	1

A score of 3 or more is highly suggestive of primary thrombocythaemia whereas a score of less than 3 suggests the presence of a reactive thrombocytosis.¹⁰¹ (* BFU-E denotes blast forming units-erythroid).

Table II Binet staging system for chronic lymphocytic leukaemia and data on median expected survival for each group of patients

Stage	Criteria	Median survival (months)
A	Less than 3 areas involved	> 120
B	Three or more areas	61
C	Haemoglobin < 100 g/l Platelets < $100 \times 10^9/l$	32

N.B. The areas assessed include the cervical, axillary and inguinal lymph nodes (whether unilateral or bilateral), the spleen and liver.

apply and is better at discriminating between patients with markedly different prognoses.

They further suggested the acceptance of standard criteria for judging response to therapy.¹⁰⁸ Half of the deaths in patients with stage A CLL are unrelated to the disease and the International Group reported on the prognostic features of stage A CLL.¹⁰⁷ Patients were subdivided into those with a lymphocyte count of $< 30 \times 10^9/l$ and haemoglobin > 120 g/l stage A'; or stage A'' if these criteria were not met. Data on over 900 patients showed that survival for patients with stage A' disease was identical to that of the rest of the population. There was also proof that patients with early disease may not require any therapy, confirming the widely held view that patients with early stage A CLL should not be subjected to treatment.

In contrast, the French study on patients with advanced CLL (stage C) has been updated and this confirmed an earlier report which showed that monthly combination chemotherapy according to the CHOP protocol gives a markedly improved 3 year survival compared to COP chemotherapy¹⁰⁹ (C = cyclophosphamide, H = adriamycin, O = vincristine and P = prednisolone). Thus adriamycin (doxorubicin) appears beneficial in patients with advanced CLL. A new drug, fludarabine, may also represent an advance in CLL, although the data are preliminary. One study showed a 50% response in the CLL, and this was achieved without undue myelosuppression.¹¹⁰ Side effects included infection and less commonly a neuropathy.

Cell lineage of Hodgkin's disease

The nature of the malignant cell (Reed Sternberg cell or RS cell) has been controversial for many years – this is partly because most cases have a relative paucity of RS cells.¹¹¹ Two approaches may help overcome this problem: firstly the isolation and purification of RS cells from lymph nodes¹¹² and secondly the application of molecular biological techniques. These latter studies depend upon rigorous criteria being imposed before a case is designated as true Hodgkin's disease; most studies are then unable to find evidence of either B- or T- cell lineage as judged by immunoglobulin or T-cell receptor beta chain gene rearrangement.¹¹³ The importance of a correct diagnosis before proceeding to genotypic analysis cannot be over-emphasized: the diagnosis of Hodgkin's disease is a *morphological* one, and if the tissue studied has been wrongly interpreted the results will be misleading. This is exemplified by papers which fail to find evidence of T-cell receptor gene rearrangement in T-cell lymphomas,¹¹⁴ and is best described using computer terminology 'garbage in, garbage out'.

However, a recent paper using a monoclonal antibody to an epitope of the T-cell receptor beta chain claimed to show *in situ* positivity in around 30% of RS or Hodgkin's cells.¹¹⁵ These data suggest that Hodgkin's disease is T-cell derived but it should be treated with caution since no control tissue appears to have been studied.

Studies using *in situ* hybridization have shown evidence of Epstein-Barr virus (EBV) DNA within RS cells from a number of patients with Hodgkin's disease,¹¹⁶ and the incidence of EBV infection is much higher in HIV seropositive patients who develop Hodgkin's disease.¹¹⁷ This is more suggestive of a B-cell lineage for the RS cell and also raises intriguing questions about the aetiology of this disease.

Iron chelation therapy

Iron overload is observed in patients who require regular blood transfusion (e.g. thalassaemia major or chronic red cell aplasia) and this leads to haemosiderosis; with cardiac failure, cirrhosis, diabetes mellitus and a slate grey skin. Patients who are untreated generally die of heart failure before the age of 25. Venesection cannot be used in these patients but effective removal of iron (chelation) with subcutaneous desferrioxamine has radically improved the survival of these patients.¹¹⁸ This treatment relies on subcutaneous infusion for 5 nights a week, year in year out. This is unpleasant for the patient and poor compliance results in early death.

Lately an oral iron chelator designated L1 has been introduced into clinical practice and early reports suggested it was safe and that it gave rise to a negative iron balance.¹¹⁹ However, doubts have been raised about the safety and efficacy of this drug:¹²⁰ one patient developed prolonged profound pancytopenia due to trilineage aplasia whilst taking L1.^{121,122} In this case the patient had absolute neutropenia for at least 17 days and she only survived her life-threatening infection because her marrow function regenerated. This complication has been observed despite less than 120 patients having been treated¹²³ and this suggests that this particular drug carries an unacceptable risk.¹²⁴ However, L1 does point to the possibility of oral chelation therapy, and it is to be hoped that a safer and effective oral iron chelator will soon be developed.

Prophylaxis of rhesus haemolytic disease of the newborn

Rhesus negative women who give birth to rhesus positive children may receive a transplacental

transfusion of red cells and this particularly occurs at term. Sensitization to rhesus D may lead to haemolytic disease of the newborn (HDN) in subsequent pregnancies and therefore rhesus negative women receive anti-D immunoglobulin at term. This mops up fetal red cells within the maternal circulation, thus reducing the incidence of rhesus sensitization to less than 10% of that previously observed. However, sensitization to rhesus D may occur during pregnancy, and give rise to new cases of HDN. A recent large study has confirmed the value of giving anti-D immunoglobulin to rhesus negative women at 28 and 34 weeks of pregnancy, as well as at term.^{125,126} This procedure has been shown to be effective in reducing the incidence of rhesus immunization and is safe.¹²⁶

Haemophilia A

Effective treatment of haemophilia A relies on the use of anti-haemophilia factor (VIIIc). However this carries the risk of transfusion-associated viral infection, particularly with human immunodeficiency virus (HIV) and non-A, non-B hepatitis.¹²⁷ The current strategy to prevent such infections relies on dissuading 'high risk' blood donors, screening blood donations and, more recently, the introduction of heat treatment. Although these approaches appear to prevent either of these particular infections¹²⁸ it can be seen that synthetic factor VIIIc would be even safer. Recombinant anti-haemophilia factor has now been prepared and successfully used in patients.¹²⁹ There was a clear therapeutic and laboratory response and neither patient developed significant antibodies.¹²⁹ The advantage of recombinant factor concentrate is the absolute certainty that it carries no contaminating viral infection. However, current technology is unable to produce factor VIIIc in clinically useful quantities.

Desmopressin (DDAVP)

This agent can be given by slow intravenous infusion and has been shown to improve haemostatic function by increasing the levels of factor VIII procoagulant and von Willebrand factor.¹³⁰ Thus it can be used in patients with mild haemophilia or von Willebrand's disease,¹³⁰ in the bleeding associated with renal failure,¹³¹ and in the post-operative situation.¹³² As DDAVP may control a haemorrhagic state without recourse to blood products it has proved extremely useful. Unfortunately there have been recent reports of acute myocardial infarction immediately following DDAVP. Therefore this drug must be used with

extreme caution in the elderly and those with arterial disease.¹³³

Rare causes of thrombocytopenia

Heparin-induced thrombocytopenia (HIT) may occur within a few days of starting heparin, when it is usually not severe and is caused by impurities within the preparation. However, late onset HIT is a life-threatening condition occurring from 8 to 25 days after starting heparin. This disorder has an immunological basis and patients have a heparin-dependent IgG antibody which induces platelet aggregation: clinically there is severe thrombocytopenia and massive thrombosis.¹³⁴ A recent case report described the successful use of intravenous immunoglobulin to reverse the thrombocytopenia and which resulted in a rapid clinical improvement.¹³⁵

Acquired amegakaryocytic thrombocytopenia is a rare condition, which may herald the onset of aplastic anaemia or myelodysplasia.¹³⁶ Cyclical amegakaryocytic thrombocytopenia is even rarer, and a recent article describes such an entity and implicates an antibody that selectively blocked the action of GM-CSF on megakaryocyte precursors.¹³⁷

Platelet transfusion therapy

A recent consensus statement by the National Institute of Health emphasized the value of such treatment in patients with thrombocytopenia attributable to chemotherapy or aplastic anaemia.¹³⁸ In contrast, platelet transfusion therapy (PTT) is generally not required in patients with consumption thrombocytopenia, since the bleeding disorder is not so severe and survival of transfused platelets is short.¹³⁹ Although a 6 unit transfusion might be theoretically expected to raise the platelet count by $60 \times 10^9/l$, in practice an increment of $40 \times 10^9/l$ is more usual.¹³⁹ Lately it has been shown that incubating platelet concentrates at 37°C for 1 hour before transfusion gives an increase in the incremental platelet count of 28%.¹⁴⁰ Thus the routine use of warmed platelets would reduce the number of platelet concentrates required by as much as 25% with considerable savings in the workload of transfusion centres. This would also reduce the number of donors to whom one patient was exposed; although it appears that this does not reduce the incidence of either alloimmunization or transmission of viral infection.^{139,141}

Tranexamic acid

This antifibrinolytic agent has been used for some years to delay clot breakdown and hence improve haemostasis. It can be given either by mouth or intravenously and is of proven value in patients who have thrombocytopenia or platelet function disorders (such as Bernard Soulier disease).¹⁴² Recent papers have drawn attention to other conditions in which tranexamic acid (TA) may be helpful.

(a) *Acute promyelocytic leukaemia (APL)*

This subtype of acute myeloid leukaemia is designated M3 and characterized by an excess of promyelocytes with cytoplasmic granules, which contain a procoagulant. These patients may present with a disseminated intravascular coagulation, which usually worsens immediately after starting cytotoxic therapy, since these leukaemic cells are lysed, thus initiating the coagulation cascade. Heparin therapy has been shown to improve survival in patients with APL.¹⁴³ More recently TA has been used successfully in such patients, leading to fewer bleeding episodes and a reduction in the number of platelet transfusions.¹⁴⁴

(b) *Anticoagulant patients*

Patients who are on long term warfarin, for example those with mechanical valve prostheses, are at risk of prolonged bleeding after dental surgery. In these patients, in whom warfarin cannot be safely discontinued, an aqueous solution of 4.8% TA used to rinse the mouth four times a day significantly reduces the number of bleeding episodes and is effective in preventing bleeding.¹⁴⁵

(c) *Thrombocytopenia*

A 4.8% aqueous solution of TA is also effective in reducing the mouth bleeding which may be seen in patients with thrombocytopenia due to bone marrow failure.

Transfusion associated graft-versus-host disease

Viable lymphocytes are transfused every time that a patient receives a blood or platelet transfusion. These lymphocytes are virtually always rejected but if this fails to happen and engraftment occurs, then donor lymphocytes will recognize the host as foreign. This will result in a transfusion associated graft-versus-host disease (TA-GvHD). Until recently this disease had only been recognized in severely immunocompromized patients, including those with severe combined immunodeficiency

disease, bone marrow transplantation (BMT), and preterm newborn infants. The disorder was also described in less than 1 in a 1000 patients receiving intensive chemotherapy for malignancy.¹⁴⁶

TA-GvHD is underdiagnosed because many clinicians are unaware of its existence and the clinical picture may be variable. However, any patient who develops aplasia within 30 days of receiving blood products may have TA-GvHD. Before last year only 70 cases had been described and all but 4 appeared to be immunocompromized. Lately Japanese workers have documented 96 cases of TA-GvHD following cardiac surgery¹⁴⁷ (an incidence of 1/660); although at present it is unresolved whether cardiac surgery is only a risk in Japanese cases, since only one case of TA-GvHD has been reported outside Japan.

TA-GvHD occurs from 4 to 30 days post-transfusion of blood products and resembles GvHD seen post-BMT in that it affects the skin, liver and gut. Typically the patient develops fever, gross erythroderma, obstructive jaundice and diarrhoea. However, in contrast to patients post-BMT, the lymphocytes are not marrow derived, and therefore recognize the host marrow as 'foreign', and this results in a profound aplastic anaemia which is rapidly fatal.

Typically, patients with TA-GvHD go downhill and die, so that chronic TA-GvHD has only been described in one patient.¹⁴⁶ Supportive therapy is generally undertaken coupled with high dose steroids or cyclosporin; this treatment is not of proven benefit. Since there is no effective therapy of TA-GvHD it is important to recognize the risk of transfusion in an individual with impaired cellular immunity. Adequate prophylaxis relies on the irradiation of blood products to 15 Gy, this dosage will prevent lymphocyte proliferation without harming red cell or platelet function.¹⁴⁶ This precaution must also be extended to blood products given to patients during bone marrow harvest for an autologous BMT, since there is a high risk of transfused lymphocytes being harvested and later giving rise to TA-GvHD.¹⁴⁸

Transmission of infection by blood transfusion

(a) *Human immunodeficiency virus (HIV)*

Transfusion of blood or factor concentrates has been associated with the transmission of HIV infection, both before the introduction of HIV screening for all blood products in 1985¹⁴⁹ and in very rare instances since then.¹⁵⁰ The prolonged incubation time from transfusion to developing full-blown AIDS (probably around 6–8 years) means that there are many individuals who are HIV seropositive but remain clinically well. At

least 500 cases of transfusion-associated AIDS have been diagnosed and a recent review estimates that a further 1500 cases of AIDS might be expected.¹⁵¹ Fortunately this figure is a large overestimate since 'post-transfusion mortality' is high. However, it does indicate that we should expect to see many more cases of transfusion-associated AIDS than we have up to now.¹⁴⁹

On a more optimistic note a large European study has looked at the incidence of HIV seroconversion in multiply transfused thalassaemic patients since 1985. Of nearly 3000 patients who were transfused with over 96,000 units of blood, no HIV sero-conversion was detected.¹⁵²

(b) Hepatitis C (HCV)

Post-transfusion hepatitis is a significant cause of morbidity and in the USA occurs after 10% of all transfusions. It accounts for 20% of all cases of acute hepatitis, and commonly leads to chronic liver disease.¹⁵³ Early work had shown that the agent responsible for most cases of 'non-A, non-B hepatitis' was a virus sensitive to organic solvents and less than 80 µm in diameter, leading to the prediction that it would be a small RNA virus.^{154,155} Using molecular biological techniques a putative virus has been isolated and a test to detect the presence of antibody developed.¹⁵⁶ This test has been shown to identify correctly the majority of cases of post-transfusion non-A, non-B hepatitis, and the virus is now designated hepatitis C.¹⁵⁶ Studies in different populations have confirmed that most post-transfusion hepatitis (PTH) is attributable to HCV.^{157,158} Further studies have been carried out in high risk populations and HCV positivity is seen in around 70–80% of haemophiliacs,^{158–160} patients with chronic PTH¹⁵⁸ and drug addicts.¹⁵⁸ Surprisingly, only a relatively small proportion of Spanish HIV sero-positive homosexuals are HCV positive.¹⁵⁸ Lately, an elegant prospective study proved that recipients of anti-HCV positive blood products had a 20-fold increased risk of developing PTH.¹⁶¹ These authors also calculated that HCV antibody testing would be 63% efficient at preventing PTH, although the test may give false negative and false positive results.¹⁶¹ Thus individuals may lose their HCV antibody positive status, although presumably remaining infectious.^{159,161} Other donors may show a false positive test, and currently the only way to confirm a positive anti-HCV test depends on proving the presence of virus within the blood: this is not possible since the viral sequence of HCV has not yet been published.

The introduction of a screening test for HCV has considerable implications for the blood transfusion services, since preliminary studies suggest that around 1% of donors are sero-positive.¹⁶²

Therefore the routine use of such a test will contract the size of the donor pool and raise the spectre of counselling individuals who are well but possess antibody to HCV. An alternative suggestion that donations should continue to be taken from these individuals but the blood should then be discarded, seems impractical.

(b) Cytomegalovirus (CMV)

CMV is carried within the lymphocytes of sero-positive individuals. CMV infection is often sub-clinical and therefore most blood products are not screened for CMV status. However, in neonates and severely immunocompromised individuals such an infection may be fatal: so that CMV negative blood products should be used.¹⁶³ An alternative strategy is to transfuse the blood products through a white cell filter and a recent study suggested a dramatic reduction in CMV seroconversion.¹⁶⁴ However, this study has been criticized as having a very high incidence of seroconversion amongst the control group and the fact that babies in the control group were of lower birth weight.¹⁶⁵

Inactivation of virus particles within blood products

Prevention of transmitted viral infection (principally non-A, non-B hepatitis, HIV and hepatitis B) is both time-consuming and relies upon identifying donors who carry potentially harmful viruses. Unfortunately such a strategy is not foolproof: for instance HIV has been transmitted by transfusion with HIV antibody negative blood on rare occasions.¹⁵⁰ Secondly, other viruses may be pathogenic but occur at such low frequency that routine donor testing is not thought to be feasible (e.g. HTLV-I: human T-cell leukaemia lymphoma virus), or their pathogenicity may not yet be recognized. Therefore an alternative strategy to prevent transmission of viral infection by blood products is to inactivate virus within either plasma and cellular blood products. There are encouraging signs that photochemical decontamination (PCD) may provide a feasible and effective way of inactivating bacteria and viruses in blood products.^{166,167} Psoralen derivatives are added to the plasma and bind to double-stranded DNA by intercalation, then on exposure to ultra-violet light the psoralen derivative covalently binds to pyrimidine bases, thereby inhibiting any further transcription or replication. This technique is proven to inactivate single or double stranded DNA or RNA viruses.¹⁶⁸

PCD has been shown to prevent the transmission of non-A, non-B hepatitis to chimpanzees transfused with treated non-A, non-B inocula. Subse-

quent challenge with the same inocula, but untreated, led to classical post-transfusion hepatitis.¹⁶⁷ Furthermore, this study was carried out on plasma which contained factor VIIIc activity and PCD did not result in a significant fall in this activity.¹⁶⁷ A further study has applied a similar technique to inactivating virus within platelet concentrates.¹⁶⁹ Other techniques of viral inactivation are also being investigated including the use of gamma irradiation and laser ultra-violet light.¹⁶⁹

New books

The speed of change in medicine coupled with the time taken for a textbook to come to fruition means that far too many are out of date by the time they arrive in the shops. However, it is worth mentioning two books published in the last 18 months. Dr

Chanarin has brought out a bench-top guide to all aspects of laboratory haematology. It is an up to date benchtop guide written by over 100 authors and is authoritative and easy to use. It is a superb book and at a cost of less than £30, it should be bought by all serious haematology departments.¹⁷⁰

The beginning of the 1980s saw a spate of new editions of the major haematology reference tomes, and these are now beginning to show their age. Therefore a new edition of 'Williams' is to be welcomed: it is 1920 pages long and from experience with previous editions it should provide a useful reference work for the 1990s.¹⁷¹

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