REVIEW ARTICLE

Recent advances in haematology

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Introduction

In the last few years there have been considerable advances in many areas of haematology, only some of which can be covered in this review. Probably on the scientific side the area of greatest new work has been in the field of stem cell culture and bone marrow function, while on the clinical side the increasing success of bone marrow transplantation is leading to a radical change in the management of many patients with haematological diseases. The development of antenatal diagnosis of haematological disorders, notably thalassaemia and haemophilia, is another rapidly expanding field. Other topics which have been selected for review are those in which there is considerable current interest and activity even if no major therapeutic advances have as yet been made. In general, discussion has been limited to work carried out in the last 2 or 3 years and recent references only are given.

Haemopoietic stem cells

The readily identifiable precursor cells of bone marrow derive from a very small population of pluripotent stem cells. These cells are not as yet themselves identifiable in man, but they give rise to committed progenitors of granulocytes and monocytes (CFU-GM), erythrocytes (BFU-E and CFU-E) and megakaryocytes (CFU-M), as well as of B and probably T lymphocytes. These committed progenitor cells are restricted to differentiation along only one or, in the case of CFU-GM, 2 pathways, but are capable of very considerable proliferation. Advances in the last 10 years have permitted their study in tissue culture by means of the colonies of mature cells they produce in agar, methyl cellulose, or plasma clot. The field of bone marrow culture has recently been reviewed by Quesenberry and Levitt (1979) and more extensively in a complete issue of *Clinics in Hematology* (1979).

Investigation of haemopoietic progenitor cells in culture has proved relevant to understanding haematological disorders characterized by abnormal proliferation as well as the aplastic anaemias. It appears that regulatory factors are produced by cells of the bone marrow stroma and act at short range to control the early proliferative phases of haemopoiesis. Thus, colony-stimulating activity (CSA), probably a product of macrophages, and burst-promoting activity (BPA), which may derive from T-lymphocytes or macrophages, are both poorly defined factors which have been shown to be essential for the *in vitro* growth of myeloid and erythroid progenitors respectively. However, the biological role of these 'activities' has yet to be substantiated (Cline and Golde, 1979).

In both chronic and acute myeloid leukaemias, growth in culture is abnormal but is still dependent on CSA. However, there is no evidence to suggest that these disorders are caused by aberrant regulatory mechanisms, since cell markers such as the Philadelphia chromosome or the isoenzymes of G-6PD have conclusively shown them to be characterized by the expansion of a single malignant clone. In polycythaemia rubra vera (PRV) and myelofibrosis, a similar approach has shown peripheral blood granulocytes as well as red cells to be derived from a single clone, which has been shown to be erythropoietin-independent *in vitro*. Nevertheless there is evidence of the presence of inhibitory factors in acute myeloid leukaemia, possibly produced by the neoplastic clone, which can suppress normal myelopoiesis, and in PRV normal erythroid colonies may be demonstrated in bone marrow culture despite their evident suppression *in vivo*. These secondary disorders of regulation would confer an important growth advantage on the malignant clone.

Recently, application of these clonal assays to the study of aplastic anaemia has suggested that,

in some cases at least, abnormalities of the micro-environment rather than stem cell deficiency may be involved. In these cases growth in culture was found to depend upon the removal of populations of inhibitory lymphoid cells, which themselves could suppress normal marrow growth. These findings are still controversial but find support from those cases where clinical recovery has been documented following therapy with immunosuppressive agents alone (Geary and Testa, 1979).

The ability to purify stem cells has been an elusive goal which may soon be a reality. Monoclonal antibodies to unique stem cell antigens are being actively sought as a means of preparing pure populations of stem cells. These might then be used for transplantation free of the risk of graft-v.-host disease.

Bone marrow transplantation

General aspects

Bone marrow transplantation has been one of the most exciting areas of development in clinical haematology in the last few years. From a purely experimental procedure it has developed into a method of treatment which may offer a hope of cure for patients with otherwise lethal diseases. A prime requirement for grafting is maximum possible histocompatibility between donor and recipient. With syngeneic (identical twin) grafts or autografts (i.e. the patient's own previously set-aside marrow) there is no immunological barrier to grafting but allogeneic grafts (grafts from a donor of different genetic make-up) involve some degree of histoincompatibility, even between HLA-matched and mixed lymphocyte culture (MLC) compatible siblings. This has two implications – firstly, that the recipient of an allogeneic graft must be immunosuppressed in order for the graft to take; secondly, that there is a considerable risk of graft-v.-host disease (GVHD) which will also require immunosuppressive drugs for its control. In marrow grafting for non-malignant conditions, e.g. aplastic anaemia, the preparation of the recipient is concerned only with immunosuppression and most such patients are prepared with cytotoxics alone, e.g. high dose cyclophosphamide; patients with immune deficiency diseases or those receiving syngeneic grafts require no preparation. Where the graft is carried out for a malignant condition, preparation is directed towards eradication of the disease as well as immunosuppression, and all patients therefore require treatment, which is usually a combination of high dose chemotherapy and total body irradiation (TBI). The more intensive immunosuppression resulting from TBI means that graft rejection is rare. The technique of transplantation involves harvesting marrow, usually 400–800 ml, from the recipient under general anaesthesia and transfusion into the recipient *via* an intravenous line

after filtration of the marrow to remove particles. The dose of cells is important, the minimum for successful engraftment being about $1 \times 10^8/\text{kg}$. There are usually signs of engraftment within 21 days after an autograft and 21–28 days after an allogeneic graft, except in aplastic anaemia where engraftment may take rather longer. Throughout this period the patient requires nursing in a sterile environment with supportive care in terms of red cell, platelet and granulocyte transfusions and rapid treatment of infection. Viral infections are more difficult to prevent than bacterial infections and varicella zoster is a particular problem in the first months after transplant (Atkinson *et al.*, 1980). Interstitial pneumonitis is a relatively common complication and, although some cases are due to viral infection, the aetiology of others is unclear, although TBI is almost certainly a factor. Interstitial pneumonitis is also more common in patients with GVHD.

The most important single cause of death after allogeneic transplantation is GVHD, a generalized syndrome affecting mainly the skin, the gastrointestinal tract and the liver. The severity ranges from rashes, mild diarrhoea and abnormal liver function tests to sloughing of the skin, uncontrollable diarrhoea and liver failure. Transient rashes are the most common and the earliest manifestation of GVHD and skin biopsy is helpful in confirmation. Attempts have been made to prevent and/or treat GVHD with methotrexate, anti-lymphocyte globulin and steroids with only minor success, although high dose methyl prednisone can modify considerably the clinical manifestations of the syndrome (Kendra *et al.*, 1981). More recently cyclosporin A has been used with considerable success in the prevention of GVHD (Powles *et al.*, 1980a), although it seems that long-term treatment is necessary as GVHD may occur when the drug is withdrawn. It is worth noting that GVHD has been described after syngeneic grafts (Rappeport *et al.*, 1979; Gluckman *et al.*, 1980) so its aetiology may not be due entirely to antigenic differences between graft and host.

Bone marrow transplantation in aplastic anaemia

Marrow transplantation was first used successfully to treat severe aplastic anaemia by the Seattle group in 1969, and has subsequently been used by several other centres, with an approximately 60% long term survival rate. The major reason for failure in most series is graft rejection which occurs in up to 60% of cases (Gordon-Smith, 1979) and which is related to two major factors, firstly that TBI is not usually employed in preparation and secondly that many patients have previously been transfused. If TBI is used, as in transplantation for acute leukaemia, then the rate of rejection is much reduced but there is a greater risk of GVHD and infection (Storb, 1980a).

Total nodal irradiation has also been used (Ramsay, 1980) in an effort to reduce rejection without increasing GVHD, but GVHD still occurred. There is evidence from renal and hepatic transplantation that cyclosporin A will reduce graft rejection (as well as its action in preventing GVHD) and preliminary evidence from the Hammersmith Hospital suggests that in aplastic patients receiving cyclosporin A, the rate of rejection is considerably reduced (Hows *et al.*, 1981).

The rejection rate is also reduced if patients are transplanted early in the disease before they have been sensitized; only 8% of such patients fail to engraft and 82% are alive and well after up to 6.5 years using cyclophosphamide alone (Storb, 1980b). Even in transfused patients the results can be improved by giving larger numbers of stem cells obtained by harvesting buffy coat as well as marrow from the donor. Using this technique the Seattle group have achieved 70% survival up to 3.5 years with a rejection rate of 16% (Storb, 1980b).

Bone marrow transplantation in acute leukaemia

The basis for allogeneic bone marrow transplantation in leukaemia is that otherwise lethal doses of chemotherapy and radiotherapy can be employed in the hope of eradicating the disease. There may also be an anti-leukaemic effect of the GVHD reaction although this remains unproved. Most centres use high-dose cyclophosphamide for preparation but some use cytosine and anthracyclines. All centres give total body irradiation but the optimal dose and scheduling is not yet clear. Initially, transplants were undertaken in end-stage patients in leukaemic relapse, and although there was enough success to justify continued work, eradication of the disease proved a major problem. The current approach is only to transplant patients in remission, either in first remission from acute myeloid leukaemia or poor prognosis acute lymphocytic leukaemia (ALL) or in second or later remission from standard risk ALL, and the results in these patients are very encouraging. In contrast to aplastic anaemia, graft rejection is very uncommon and the major cause of death is GVHD with or without interstitial pneumonitis; such deaths are commoner in older patients. Recurrence of leukaemia is also a minor problem in the long term. Thus the latest results from Seattle show that of 48 patients with acute non-lymphocytic leukaemia transplanted in first remission 65% are living in complete remission up to 4 years after transplant with only one recurrence (Thomas *et al.*, 1979a; Thomas, 1980) and similar good results have been obtained at other centres (Royal Marsden, Paris, Minneapolis, Duarte) (Powles *et al.*, 1980b; Blume *et al.*, 1980). Powles *et al.* (1980b) at the Royal Marsden Hospital compared the results of transplant

in 22 patients in first remission from AML with 28 similar patients who did not have donors and who received maintenance chemo-immunotherapy alone. In the transplant group, 4 died of GVHD or interstitial pneumonitis and 2 died of recurrent leukaemia; the remaining 14 patients (64%) remain alive and well up to 3 years after transplant. In the non-transplant group, 12 of the 28 have died, 11 of recurrent leukaemia and 1 of infection, and only 29% remain alive and in complete remission. Thus, in young adults with AML in first remission, transplant offers a longer leukaemia-free survival than chemotherapy, and relapses after 2 years are rare, suggesting the possibility of long-term cure. The results in ALL are not quite as encouraging because the frequency of relapse appears to be higher (Thomas *et al.*, 1979b), although at the Westminster Hospital 11 children with ALL in second remission have had transplants and 9 of these are alive and well, in complete remission with a median survival of 280 days; there were 2 deaths from GVHD but no relapse (A. J. Barrett, personal communication).

Even if transplantation is shown to be the optimum treatment for the majority of patients not more than one in 4 will have an HLA MLC compatible donor. For the remaining patients there is the possibility of autografting marrow harvested in complete remission and preserved until the patient relapses (Dicke *et al.*, 1979). It is theoretically possible to free the harvested remission marrow from residual leukaemic cells by exploiting differences in antigenic composition, cell size density or other properties. The patient can then be treated with cytotoxics and TBI while still in remission and autografted with the decontaminated marrow. Work is already beginning along those lines and may ultimately offer a hope of cure to much larger numbers of patients than can be cured by allogeneic bone marrow transplantation.

Other disorders

The role of bone marrow transplantation in the management of chronic granulocytic leukaemia is discussed below. Other haematological diseases in which bone marrow transplantation may ultimately provide a cure include thalassaemia and sickle cell disease, and there is a wide scope for the use of marrow transplantation in the management of non-haematological malignancies.

The leukaemias

Acute lymphoblastic leukaemia in childhood

In the early 1970s considerable advances were made in the treatment of ALL, notably with the development of an effective regime for cranio-prophylaxis by Pinkel's group in Memphis (Aur *et*

al., 1978). The treatment of ALL became fairly standard, with induction, usually with vincristine and prednisone alone, followed by cranioprophylaxis with cranial irradiation and intrathecal methotrexate and then 2 to 3 years' maintenance therapy with oral drugs, usually methotrexate and 6-mercaptopurine. With this type of management, approximately 50% of children achieve long-term remission and probably cure. However, this means that 50% still die from the disease, the majority in haematological relapse, and so there is considerable scope for improving the results. The chief problem is to give more effective anti-leukaemic therapy to those who need it without giving unnecessarily toxic treatment to those who would already achieve long-term survival. It has become apparent that some groups have a worse prognosis than others, e.g. patients with T or B cell disease and those presenting with a high blast cell count, and that more intensive and/or prolonged therapy is necessary for those children. Some of the 'standard risk' group might also benefit from more intensive therapy in terms of antileukaemic effect but the dangers of increased toxicity are not negligible; even at present, 5–10% of children die in remission from viral and other infections.

It is now widely accepted that the duration of first complete remission is markedly affected by the initial chemotherapy and that the addition of a third drug, usually asparaginase, to vincristine and prednisone during induction prolongs the duration of remission (Mauer, 1980). The use of more intensive induction regimes containing prolonged asparaginase or anthracyclines is being explored both by the MRC and in the U.S.A. and the value of a period of intensive consolidation after induction is also under study. The standard regime of 2400 rad cranial DXR combined with intrathecal methotrexate for cranioprophylaxis is still widely used and is undoubtedly effective in preventing CNS relapse. However, anxieties have arisen over long-term toxicity: intellectual performance is impaired to some extent and abnormalities have been shown on CAT scans in asymptomatic patients (Eiser, 1978; Peylan-Ramu *et al.*, 1978). Intellectual development may be particularly impaired in children receiving cranial DXR in the first 2 years of life and it is recommended that very young children should instead receive additional intrathecal chemotherapy.

With regard to maintenance chemotherapy, many schedules now include intermittent 'pseudo-reinduction' therapy with intravenous cytotoxics in addition to oral 6-mercaptopurine and methotrexate (Mauer, 1980). This approach seems to increase the number of long-term remissions and may reduce the frequency of testicular relapse in boys. However, the use of very intensive chemotherapy throughout

maintenance is associated with considerable immunosuppression and increased deaths from infection. The optimum duration of therapy is between 2 and 3 years after which time the risks of therapy outweigh the risk of relapse, which is less than 10% (George *et al.*, 1979). Bone marrow relapse occurring after cessation of therapy does not necessarily hold the same poor prognosis as relapse occurring during therapy since long-term survival is still possible (Rivera *et al.*, 1979). However, if relapse occurs during therapy there is virtually no chance of long-term survival (Cornbleet and Chessels, 1978), and so more patients with ALL in second remission are now being referred for bone marrow transplantation (*v.s.*).

Another problem of current interest is that of testicular relapse in boys, which probably accounts at least partly, for their inferior prognosis (Editorial, 1978). Although isolated testicular relapse can be successfully treated without altering the chances for long-term cure (Wong *et al.*, 1980) there is little doubt that reseeding can occur from testis to marrow with resulting haematological relapse. Testicular biopsy at the time of stopping therapy reveals that 10–20% of boys have infiltration (Eden *et al.*, 1978; Wong *et al.*, 1980); these children are treated with irradiation and further long-term chemotherapy with encouraging results. However, some of those with negative biopsies will subsequently develop testicular relapse and frequent biopsies are impracticable. A trial of prophylactic testicular irradiation has been carried out by the MRC but as yet it is too early to evaluate the results, and at present it is probably advisable to reserve this for the poor prognostic group.

Acute non-lymphocytic leukaemia

Since the dramatic improvement in remission rate in the early 1970s following the introduction of cytosine and the anthracyclines, there has been little advance in the chemotherapy of this disease. The complete remission rate remains at around 70% overall, with a mean remission duration of about one year and a mean survival of 12–18 months. Long-term survival is extremely rare. Various new approaches are under study including varying schedules for remission induction, the role of a period of intensive consolidation, and the optimum type and duration of maintenance therapy (Gale, 1979) but as yet no significant improvements have emerged and, at present, early bone marrow transplantation appears to offer a better prognosis than any form of chemotherapy (*v.s.*).

Chronic granulocytic leukaemia

The increased use of cytogenetic and marker studies has led to a clearer understanding of the

evolution of this disease. In the chronic phase, over 95% of cases are found to have the Philadelphia (Ph⁺) chromosome, a 22 chromosome with loss of the chromatin material from the long arms which in the majority of cases is translocated to a no. 9 chromosome. However Ph⁻-negative cases undoubtedly do exist; they often show atypical features and tend to have a worse prognosis. Almost all patients ultimately develop a more acute form of the disease; the terms 'acute transformation' or 'metamorphosis' are now used to describe this stage rather than 'blast-cell crisis' since it is not always characterized by an increase in numbers of blast cells (Spiers, 1979). Transformation may take the form of an accelerated myeloproliferative phase, an acute leukaemic picture, extramedullary tumours or simply deteriorating health with a failure to respond to previously successful treatment. Cytogenetic studies show that there are often additional chromosome abnormalities of which a second Ph⁺ chromosome is the most common. Where there is an acute leukaemic picture, marker studies demonstrate that although in 60% of cases the phenotype is that of AML, in 30% of cases the blast cells show similarities to those of common ALL (ALL-positive, TDT-positive), suggesting that this cell is a stem cell common to the lymphoid and the myeloid series (Marks, Baltimore and McCaffrey, 1978).

The therapy of the chronic phase has advanced little; drugs such as hydroxyurea, dibromomannitol and melphalan and intermittent high dose busulphan or cyclical chemotherapy are as effective as continuous busulphan in control of the chronic phase but do not delay the onset of transformation. However, melphalan is a shorter-acting drug than busulphan and carries less danger of irreversible hypoplasia. Hydroxyurea may be useful in the accelerated phase before overt transformation, where busulphan or melphalan may no longer be effective. More intensive chemotherapy has been used but although karyotypic conversions have been obtained in some cases the overall survival has not been significantly improved (Sharp *et al.*, 1979). Neither does early elective splenectomy delay the onset of metamorphosis (Ihde *et al.*, 1976) although it may make the terminal phase easier to manage. Several successful marrow transplants have been carried out in the chronic phase from an identical twin donor with eradication of the Ph⁺ clone (Fefer *et al.*, 1979) and it seems probable that as techniques improve, allogeneic transplantation will be the next step.

Once the patient has entered metamorphosis, even the most intensive chemotherapy is of little benefit unless the blast cells show lymphoid features, when about 50% will obtain a remission using regimes containing vincristine and prednisone (Rosenthal *et*

al., 1977; Marks *et al.*, 1978). This bleak outlook has led to the development of autografts, in which peripheral blood stem cells harvested during the chronic phase and cryopreserved are used to re-constitute the marrow after ablative chemotherapy with or without high dose TBI (Goldman, 1978; Goldman *et al.*, 1979). It is easy to restore marrow function in these patients but unfortunately the duration of the second chronic phase tends to be rather short, probably because it is impossible to eliminate refractory blast cells (as shown by the disappointing results of identical twin transplant at this stage). However, it does offer some hope until allogeneic transplantation for chronic phase CGL is established.

Haemophilia and von Willebrand's disease

In the last few years there has been a considerable advance in the understanding of the structure and function of Factor VIII, which has led to improved diagnostic accuracy of the Factor VIII-deficiency diseases, to the diagnosis of carriers of haemophilia and more recently to preliminary work in the antenatal diagnosis of haemophilia.

Factor VIII is a glycoprotein of large molecular weight which has at least 3 distinct properties – procoagulant activity (Factor VIII_C), Factor VIII related antigenic activity (Factor VIII_{RAg}), detected by precipitation with rabbit antiserum, and ristocetin co-factor activity (Factor VIII_{RCOF}). The latter is the property of Factor VIII which is necessary for platelets to aggregate in the presence of the antibiotic ristocetin, and as it is absent in von Willebrand's disease it may also be called Factor VIII_{vwt}. The relationship between these 3 properties is not yet clear; they may be 3 facets of the same molecule, or Factor VIII_C may be a small molecule which is carried on the larger Factor VIII_{RAg}. *In vivo* the related substances do not always occur together and the different properties can be separated by *in vitro* techniques (Austen, 1979).

Factor VIII_{RAg} and VIII_{RCOF} are closely related. Both are synthesized by vascular endothelial cells and possibly also by megakaryocytes and platelets (Bloom, 1979). The site of VIII_C synthesis is still unclear, although it seems to be in some way dependent on the presence of Factor VIII_{RAg}. In haemophilia, Factor VIII_C is reduced or absent but Factor VIII_{RAg} and VIII_{RCOF} are normal, and synthesis of VIII_C therefore seems to be under X chromosome control. In classical von Willebrand's disease all the properties of Factor VIII are reduced but Factor VIII_C can be synthesized if the patient is transfused with normal or haemophilic plasma which contains Factor VIII_{RAg}. These observations suggest that the low VIII_C in von Willebrand's disease is secondary to the low VIII_{RAg}, and that

VIII_{RAG} and VIII_{RCOF} are under autosomal control. The genetic aspects of Factor VIII have been reviewed by Graham (1980). Recently, a further property of Factor VIII was described. Factor_{CAG} or coagulation associated antigen which is closely related to Factor VIII_C but is stable and present in serum. It is detected by precipitation using human antiserum, in contrast to VIII_{RAG} which is detected with rabbit antiserum (Peake and Bloom, 1978).

Classical haemophilia is diagnosed by the finding of a low VIII_C (less than 40% of normal) in the presence of a normal or high VIII_{RAG} and normal platelet aggregation with ristocetin (i.e. normal VIII_{RCOF}). The discrepancy between VIII_C and VIII_{RAG} concentration can be used in the detection of carriers of haemophilia whereas an VIII_C measurement alone is virtually useless since the carrier range considerably overlaps the normal. Using family data and VIII_C : VIII_{RAG} ratio, a probability can be arrived at for predicting carrier status (Graham, 1979). In general, a woman with a family history and an VIII_C : VIII_{RAG} ratio of 0.7 or lower is likely to be a carrier. Where a woman who is a known or probable carrier becomes pregnant it may now be possible to determine whether a male fetus is normal or haemophiliac. Factor VIII is synthesized from an early stage and can be detected in fetal blood sampled at fetoscopy at 20–22 weeks following amniocentesis to determine the fetal sex. Preliminary results show that it is possible to distinguish clearly between normal and haemophiliac fetuses by assay of plasma VIII_C and that it is possible to diagnose haemophilia antenatally with a high degree of accuracy (Mibashan *et al.*, 1979; Firshein *et al.*, 1979). Some of the problems relate to the difficulty of obtaining a sample which has not been activated by amniotic fluid and the newer technique of Factor VIII_{CAG} assay may prove particularly useful in antenatal diagnosis (Peake *et al.*, 1979). It must, of course, be borne in mind that many parents of haemophiliac children will choose either to have no further children or to continue the pregnancy regardless of the risk rather than have a late abortion (Evans and Shaw, 1979).

The treatment of haemophilia has also improved in recent years with the development of freeze-dried Factor VIII concentrates. These allow the administration of high doses of Factor VIII in a small volume, and more importantly facilitate treatment at home or at work, allowing haemophiliac patients much greater mobility and independence from the hospital (Ingram *et al.*, 1979). Unfortunately, there is still insufficient NHS concentrate available to meet demand, and in 1979 only one-third of all concentrate used was NHS concentrate. The remaining requirement has to be met by commercial concentrate which is extremely expensive and there is no

doubt that many more patients could benefit from home therapy if more NHS concentrate were available. The main limitation in its production at present is inadequate supply of plasma from the Blood Transfusion Centres to the fractionating centres, and to a lesser extent, inadequate production facilities (Editorial, 1978), and attempts are being made to improve the situation.

With current therapy the majority of haemophiliacs grow up without deformity and with relatively few hospital admissions. However, the use of large amounts of blood products, in particular freeze-dried Factor VIII concentrates, has led to an increasing incidence of chronic hepatitis in haemophiliacs (Seeft and Hoofnagle, 1977; Preston *et al.*, 1978). The incidence of abnormal liver function tests is as high as 50% in some series, and these abnormalities have been shown to persist in many patients. A significant proportion of such patients have serological evidence of HB_s infection but this does not account for all the cases, and chronic infection with non-A and non-B hepatitis virus is presumably also important. Most of the patients are asymptomatic and the importance of abnormal liver function tests in such patients is unclear. Liver biopsies have confirmed chronic liver disease in some patients with raised transaminases (Preston *et al.*, 1978) but the frequency of structural liver damage is not known, largely owing to the difficulty of performing liver biopsy in haemophiliac patients. Until further data become available no treatment is recommended for asymptomatic patients but, where there is progressive deterioration in liver function, steroids have been used with some benefit.

Classical von Willebrand's disease is diagnosed by a reduced VIII_C, VIII_{RAG} and reduced or absent platelet aggregation with ristocetin (i.e. reduced VIII_{RCOF}). The reduced platelet aggregation with ristocetin *in vitro* appears to be related to an *in vivo* defect in adhesion of platelets to vascular subendothelium (Nilsson and Holmberg, 1979) and this is responsible for the prolonged bleeding time. The platelets themselves are normal and will react normally if suspended in normal plasma or after transfusion of the patient with Factor VIII. The level of Factor VIII_C in von Willebrand's disease is not usually as low as in haemophilia and the bleeding manifestations are more often due to the impaired platelet function, i.e. the patients complain of menorrhagia, bruising and mucous membrane bleeding rather than joint or muscle bleeds. Some patients may not have all the features of classical von Willebrand's disease: for example, the level of VIII_{RAG} may be normal (it may however be qualitatively abnormal as shown by abnormal electrophoretic mobility). Other patients may have reduced VIII_C and VIII_{RAG} but still have enough normal

VIII_{RAG} and VIII_{RCOF} for the platelets to behave normally, i.e. they have a normal bleeding time and normal platelet aggregation with ristocetin. Such cases are termed variant von Willebrand syndromes (Nilsson and Holmberg, 1979; Zimmerman, Abildgaard and Meyer, 1979).

Platelet function and antiplatelet drugs

In the last few years there has been a great advance in our understanding of normal and abnormal platelet function, in particular the elucidation of the role of prostaglandins in platelet aggregation. This is the subject of a recent review by Moncada and Vane (1979). In normal haemostasis, platelets adhere to collagen or sub-endothelial microfibrils, these 2 properties being related to *in vitro* aggregation by collagen and ristocetin respectively. The platelets then undergo a marked shape change and release various chemicals including ADP, ATP, 5-HT and fibrinogen, which cause platelet aggregation. Some of these chemicals, in particular ADP, are themselves powerful inducers of the release reaction and exert a positive feedback action causing further platelet aggregation. If the release reaction is inadequate, however, the platelets dissociate again and regain their normal shape. There is also a negative feedback pathway: some of the ATP released is converted to cyclic AMP by the enzyme adenylyl cyclase and this cyclic AMP directly inhibits platelet aggregation (Fig. 1).

The initial change in the platelet membrane activates the enzyme phospholipase A with the release of arachidonic acid from the membrane. This is then converted by cyclo-oxygenase to a cyclic endoperoxide, PGG₂, which is subsequently converted either to prostacyclin (PGI₂) or to thromboxane A₂. In

platelets, the main product is thromboxane A₂ which is a potent inducer of the release reaction, and which also causes vasoconstriction. Aspirin inhibits the enzyme cyclo-oxygenase, reducing synthesis of thromboxane A₂ and hence interfering with the release reaction and further aggregation. The vessel walls also possess prostaglandin synthesizing enzymes but here the main product of cyclic endoperoxide is PGI₂; the cyclic endoperoxide generated by adhered platelets can also be metabolized in the vessel wall to PGI₂. PGI₂, in contrast to thromboxane A₂, is a vasodilator and inhibitor of platelet function since it potentiates the action of adenylyl cyclase and so increases platelet cyclic AMP levels. The balance between the generation of thromboxane A₂ and PGI₂ is obviously vitally important for the regulation of platelet function. There is some evidence that this balance may be modified by dietary fatty acids, for example, a diet rich in arachidonic acid enhances platelet reactivity while dietary eicosapentaenoic acid has the reverse effect, and Greenland Eskimoes, whose diet is rich in the latter, have a bleeding tendency with a very low incidence of atherosclerosis (Editorial, 1980; Moncada and Vane, 1979). In the rare and ill-understood syndrome of thrombotic thrombocytopenic purpura and in the haemolytic-uraemic syndrome there is preliminary evidence that the platelet aggregation and thrombus formation may result from reduced prostacyclin synthesis by the vessel wall (Remuzzi *et al.*, 1978). No doubt further disturbances of the platelet/vessel prostaglandin balance will be identified in the near future.

The chief antiplatelet drugs used therapeutically for their antiplatelet action are aspirin, sulphinyprazole and dipyridamole, although a wide variety of other drugs interfere with platelet function,

FIG. 1. Platelet function in relation to prostaglandins and antiplatelet drugs. ⊕, positive feedback; ⊖, inhibition; ASA, acetyl salicylic acid; DP, dipyridamole.

including many anti-inflammatory drugs and tricyclic antidepressants. The mode of action and the clinical use of antiplatelet drugs have recently been reviewed by Weiss (1978) and Roden and Kendall (1978). Aspirin irreversibly inhibits cyclo-oxygenase by acetylating it at its active site. Both thromboxane A_2 and PGI_2 synthesis are therefore reduced but the major effect is on the platelets so that platelet thromboxane A_2 is reduced to a greater extent than vessel wall PGI_2 and the net result is of platelet inhibition. Sulphinpyrazone is a competitive inhibitor of prostaglandin synthesis and inhibits the release action. Dipyridamole inhibits phosphodiesterase with a resultant increase in platelet cyclic AMP which inhibits the release reaction. The dose producing significant inhibition of platelet function frequently causes side effects such as flushing so that it is often used at a lower dose in combination with aspirin.

Not all the drugs are of equal effectiveness in a given situation, and it has been to some extent arbitrary which drugs have been adequately tested in any particular disease. Aspirin has been shown to be effective in reducing the frequency of transient ischaemic attacks (Fields *et al.*, 1977) and the incidence of associated strokes and death, at least in men (Canadian Cooperative Study Group, 1978). It has also been shown to reduce the incidence of venous thrombosis and pulmonary embolism after hip surgery; the effect may be more pronounced in men. The role of aspirin in ischaemic heart disease remains controversial. There is some evidence that it reduces the risk of recurrent infarction in the first year after a myocardial infarct in men but this is not conclusive and multicentre trials are still in progress. Sulphinpyrazone has also been shown as reducing the frequency of sudden death in the first year after myocardial infarct (Anturane Reinfarction Trial Research Group, 1978). Other proved benefits of sulphinpyrazone include a marked reduction in incidence of venous thrombosis in patients with recurrent deep vein thrombosis (Steele, Ellis and Genton, 1978) and a reduction in thrombosis of arteriovenous shunts in patients on dialysis. However, sulphinpyrazone appears to be of little benefit in patients with transient ischaemic attacks (Canadian Cooperative Study Group, 1978). Dipyridamole seems to be the least useful of the 3 drugs: it is ineffective in the prevention of venous thrombosis or transient ischaemic attacks and there is no evidence that it is of use in ischaemic heart disease. In combination with warfarin, however, it increases platelet survival and reduces the frequency of embolization in patients with prosthetic heart valves.

There are obvious gaps in the clinical data and discrepancies to be explained. For example, why is

sulphinpyrazone of benefit in recent myocardial infarction and recurrent venous thrombosis but not in cerebral ischaemia, and why does aspirin seem to be of more benefit in men than in women? Further research into the mechanisms and sites of action of these drugs and more extensive clinical trials may provide the answers.

The thalassaemia syndrome

Major advances have taken place in the last few years both on the scientific and the clinical side. The development of techniques for DNA hybridization and for gene dissection and mapping have led to a greater understanding of the range of defects in the thalassaemia syndromes. In α -thalassaemia there is complete deletion of one or both haploid α -chain genes while in β -thalassaemia it is the β messenger RNA which appears to be absent or inactive in the presence of intact β -chain genes (Weatherall, 1977; Forget, 1979). Increased knowledge of the mechanisms controlling the switch from γ -chain to β -chain synthesis during fetal development may ultimately lead to the ability to reverse this process in β -thalassaemia, promoting the synthesis of red cells containing high concentrations of HbF and reduced amounts of the damaging free α -chains.

At present, patients with β -thalassaemia major die in their late teens of iron toxicity resulting from chronic blood transfusion. The use of intramuscular desferrioxamine at maximal tolerated doses combined with intravenous administration at the time of transfusion is unable to chelate all the additional iron although it may be possible to hold the patient in fairly stable positive iron balance. The development of a technique for continuous subcutaneous infusion, usually administered for 8–12 hr overnight, has made it possible to chelate much larger amounts of iron than with the same dose of desferrioxamine given i.m. (Propper *et al.*, 1977). Chelation is enhanced still further by oral ascorbic acid (Hussain *et al.*, 1977). Using continuous subcutaneous desferrioxamine it is possible to convert even heavily iron-loaded patients to negative iron balance. Moreover, it seems that while i.m. desferrioxamine only begins to chelate iron where there is already a significant iron load, subcutaneous desferrioxamine is effective right from the start of a transfusion regime (Modell, 1979; Jacobs, 1979). More effective chelation means that there is less risk to patients of employing a high-transfusion regime, which by maintaining the haemoglobin between 10 and 14 g/dl has been shown to prevent bony deformity, malocclusion of teeth, hepatosplenomegaly and poor growth usually associated with thalassaemia major (Modell, 1977). Hitherto the effects of iron toxicity have begun to be apparent at about 13 years of age with delay in the onset of puberty, but it is hoped that the combination

of a high-transfusion regime with subcutaneous desferrioxamine will allow the normal development of these children and prolong their survival at least into their twenties and thirties.

Another major advance in the last few years has been the development of antenatal diagnosis of the haemoglobinopathies. Some HbA is present even in early fetal life, i.e. β -chain synthesis is occurring, and it is possible with a small fetal blood sample to determine by isotopic methods whether normal amounts of β -chain and HbA are being produced, and also if HbS rather than HbA is being synthesized (Editorial, 1977). Early attempts to obtain fetal blood by placental needling or aspiration through a fetoscope were associated with considerable risk to the fetus and there were also some misdiagnoses; however, sampling techniques and diagnostic accuracy have greatly improved and it is now possible to diagnose β -thalassaemia major with over 95% accuracy, with a fetal wastage of 5–10% (Kan *et al.*, 1980; Cao *et al.*, 1980; Alter, 1980). With antenatal diagnosis it may be possible to eradicate the disease in restricted populations and greatly to reduce its overall incidence. One problem is that it is not possible to obtain fetal blood until 18–20 weeks' gestation and some women find it difficult to accept termination once the pregnancy has reached this stage. However, therapeutic abortion is more acceptable in thalassaemia, which is still a lethal disease, than it is for example with haemophilia (v.s.).

Vitamin B₁₂, folate and megaloblastic anaemia

Vitamin B₁₂ transport proteins

Three proteins binding B₁₂ are present in human plasma. Of these, only one, transcobalamin II, has been shown to be physiologically important. It is a small molecular weight protein (36 000) that is essential in moving B₁₂ in and out of cells. Its congenital absence, or, more likely, the presence of a form not binding B₁₂, leads to a severe megaloblastic anaemia in the first few months of life responding only to large doses of B₁₂ given every few days. It is produced in the liver, macrophages, intestinal cells and probably several other tissues as well. Several TC II isoproteins can be demonstrated by electrophoresis combined with autoradiographs, and one rare variant is associated with impaired B₁₂ transport and a megaloblastic anaemia (Frater-Schröder, Hitzig and Büttler, 1979).

Nutritional vitamin B₁₂ deficiency

The large emigration of Indians from East Africa to the United Kingdom has enabled us to see a new variant of B₁₂ deficiency. These Indians are Hindus and are (or were) strict vegetarians taking very little or no milk, no cheese, no eggs and no meat. Under

these circumstances the only B₁₂ in their diet arises from bacterial contamination of otherwise B₁₂-free food. Megaloblastic anaemia due entirely to a dietary lack of B₁₂ is common at all ages and both sexes and responds fully to oral B₁₂, say 5 μ g, daily. The absorption of B₁₂ is normal in nutritional B₁₂ deficiency. Virtually all Hindu vegetarians, including those with entirely normal blood and marrows, have low serum B₁₂ concentrations.

Nitrous oxide and vitamin B₁₂ (Chanarin, 1979; Chanarin *et al.*, 1980)

Laughing gas (N₂O) is activated and cleaved by the organo-metallic bond in B₁₂ which, in turn, is oxidized to an inactive form. Prolonged inhalation of N₂O by man leads to a megaloblastic anaemia, and long-term addiction leads to a neuropathy, like subacute combined degeneration of the cord.

Biochemical inactivation of B₁₂ stops the synthesis of methionine from homocysteine. This in turn has 2 important consequences.

1. There is a fall in supply of *S*-adenosylmethionine which is an overall methylating reagent. Failure to methylate proteins in the white matter (Crang and Jacobson, 1980) is probably the cause of B₁₂ neuropathy.

2. The oxidation of the methyl group of methionine to formate also declines. Some 60–80% of methionine methyl follows this path. Formate is required to make formylfolate and to provide a carbon unit for thymidine synthesis. Failure of the latter path leads to megaloblastic anaemia in man.

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