Wiskott-Aldrich syndrome: new perspectives in pathogenesis and management

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The Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterised by thrombocytopenia, immune deficiency, eczema and susceptibility to malignant disease. Other features include recurrent non-septic arthritis, asthma, bloody diarrhoea, and nephropathy. Although rare, with an incidence of four per million live male births [1], this disorder has been the subject of intense study by haematologists, immunologists and paediatric oncologists since it was first described by Wiskott in 1937 [2]. In the fully expressed form, the diagnosis is evident within the first year of life and, until recently, has been almost invariably associated with a fatal outcome in infancy or early childhood. Investigations are beginning to shed light on the nature of the cellular and molecular defects responsible for the clinical manifestations and significant progress has also been made in the practical management of WAS patients. Effective methods of carrier detection have yet to be developed, but recent localisation of the WAS gene using DNA analysis should provide the first step towards achieving this goal. A description of the advances in these areas forms the basis of this article.

Immune deficiency is a consistent feature in WAS, although the expression in individual patients is variable. Commonly, there is impairment of cell mediated immune function manifested by absent delayed hypersensitivity reactions on skin testing and reduced circulating T cells with diminished mitogen and antigen specific responsiveness [3,4]. T-helper/T-suppressor cell ratios are abnormal with a reduction of T-helper cells [5]. Humoral immunity is also often impaired and may, in part, reflect the underlying T cell dysfunction. Early studies demonstrated the frequent absence of isohaemagglutinins and hypercatabolism of immunoglobulins [6,7]. Patients characteristically have low serum IgM with raised IgE and levels of IgA and IgG which are either normal or elevated. Transient paraproteinaemias in low concentration have also been reported [8]. The specific antibody response to capsular polysaccharide antigens is frequently impaired; it predominantly involves the IgG_2 subclass and is thought to be thymus independent [4]. This implies that a specific B cell defect or abnormality of antigen processing is present. However, depressed responses to T cell dependent antigens have also been described [4] and *in vitro* evidence of impaired T-helper cell function [9] supports the notion that disturbances of T cell regulation contribute to the abnormalities of humoral immunity.

Lymphocytes from patients with WAS have recently been shown to have altered expression of a surface sialoglycoprotein (sialophorin) of molecular weight 115,000 present on normal lymphocytes and monocytes [10,11]. In all eight patients studied, the glycoprotein was either absent or reduced and in an abnormal molecular form with incomplete sialylation. Sialophorin contains many O-linked disaccharide units which are structurally similar to the carbohydrate chains of platelet glycoprotein GP1b and erythrocyte glycophorin. In view of the known abnormalities of platelet glycoproteins in WAS (see below), it has been speculated that the underlying molecular defect resides in the synthesis or degradation of the common carbohydrate components of these surface structures [10]. The function of sialophorin is unknown but might be expected to be important in cell-cell interactions or antigen recognition and processing. An alternative and novel interpretation is that the surface glycoprotein is involved in the regulation of normal cell senescence and that its absence in WAS leads to acceleration of this process [11].

Clinical manifestations of the immune deficiency commonly take the form of skin abscesses, otitis media and chronic sinusitis, interspersed with severe life-threatening pneumonia, meningitis and septicaemia. Staphylococcus, pneumococcus, pseudomonas and systemic fungal infections are frequently encountered and susceptibility to protozoan infections including *Pneumocystis carinii* pneumonia is also well recognised [12]. Cytomegalovirus (CMV), measles virus and herpes simplex virus infections may be fulminant, but disseminated varicella has been effectively treated with adenosine arabinoside and zoster hyperimmune globulin [5]. Although initial reports suggested that transfer factor was of clinical benefit in restoring immune function [13,14], this agent may accelerate the nephropathy seen in a proportion of patients with WAS [15] and is not generally considered to be of major therapeutic value. Many authorities now agree, however, that immunoglobulin replacement therapy has a definite place in the management of selected patients

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and significantly reduces the frequency of septicaemias and recurrent pyogenic infections [16]. Anecdotal evidence suggests that this treatment may also improve eczema and increase platelet counts [17,18]. The availability of intravenous gammaglobulin eliminates the risk of local reactions and haematoma associated with the intramuscular preparation. Dose levels required in individual patients are difficult to anticipate and must be determined according to clinical response, but are generally in the range 0.1–0.4 g/kg body weight administered every 2–4 weeks.

A moderate to severe bleeding tendency due to thrombocytopenia and defective platelet function represents a major complication for patients with WAS. Though expression of the platelet abnormality is again variable, many patients are at risk of life-threatening intracranial, pulmonary or gastrointestinal haemorrhage. The thrombocytopenia is predominantly due to increased platelet destruction [19], although impaired platelet production may also be a factor [4]. A reduction in platelet volume is typical [19] and abnormalities of ultrastructure have also been described [20]. The shortened survival of autologous platelets [19,21] and the restoration of normal platelet number and size following splenectomy [22] suggests that the thrombocytopenia mainly results from splenic sequestration of defective platelets with perhaps selective removal of larger forms. In some patients, sudden rapid falls in platelet count associated with elevated platelet bound IgG has raised the possibility of a superimposed immune mediated thrombocytopenia [22] and splenectomised individuals may be particularly susceptible to this complication [23]. Platelet function is impaired in terms of diminished aggregation in response to adrenaline, thrombin, collagen and ristocetin [21,24]. Other reported abnormalities of WAS platelets include absence of the storage pool of adenosine nucleotides [21] and impaired mitochondrial ATP regeneration [25]. The surface glycoproteins GP1a and also GP1b, the von Willebrand factor receptor involved in platelet adhesion, are markedly diminished as shown by SDS-PAGE electrophoresis [24] or, more simply, by specific monoclonal antibody binding using a fluorescent activated cell sorter (Macartney and Standen: unpublished data).

In clinical practice, it is important not to administer inadvertently drugs which impair platelet function. Measurement of the half-life of transfused allogeneic platelets has yielded conflicting results [4,26,27], but most studies suggest that they have near normal survival and random donor platelet concentrates are haemostatically effective for prophylaxis and following haemorrhagic complications. Nevertheless, in serious bleeding it is prudent to confirm satisfactory one-hour platelet increments. If available, red cell and platelet concentrates from CMV-negative donors are preferable for transfusion and the risk of graft versus host disease (GVHD) mediated by donor lymphocytes should be eliminated by using irradiated blood products. More radically, splenectomy consistently produces a rapid and sustained rise in platelet count and may be an unavoidable step in serious bleeding. In many patients, there is long term attenuation of the bleeding tendency and associated risk of fatal haemorrhage. Although early studies emphasised the detrimental effect of splenectomy on the pre-existing immune deficit [28], more recent information indicates that prophylactic antibiotics can ameliorate the enhanced susceptibility to bacterial infection [22]. With the additional precaution of regular prophylaxis with intravenous immunoglobulin, this procedure should now be considered a relatively safe therapeutic option in patients particularly prone to haemorrhagic complications.

Bone marrow transplantation has recently provided a more fundamental approach to the correction of both the haematological and immune defect in WAS. Early attempts using high dose cyclophosphamide as a conditioning agent led to lymphoid reconstitution but failure to correct the platelet abnormality [29]. Although compatible with long-term survival [30], more effective conditioning usually involving combination therapy with cyclophosphamide and busulphan has consistently led to full engraftment [31]. In a review of a series of HLA-matched sibling donor transplants in WAS patients from various centres up to 1984, O'Reilly [32] has stressed the mild degree of GVHD commonly encountered and the excellent survival data now accumulating. The procedure is established as the treatment of choice if an appropriate donor is available and should probably be performed at the earliest opportunity in the course of the disorder. However, transplantation is currently applicable to only a minority of patients because most do not have a suitable matched sibling donor and unmatched marrow grafts frequently result in severe GVHD. Nevertheless, some encouragement can be drawn from preliminary studies which suggest that reconstitution with haploidentical or partially mismatched marrow may be feasible if more aggressive immunosuppression is used in conjunction with marrow purging to eliminate donor T cells and their precursors [33]. A similar approach has certainly proved effective in severe primary T cell deficiencies where haploidentical marrow grafting is regularly successful following T cell depletion and is associated with a low incidence of GVHD [34].

The increased susceptibility of patients with WAS to malignancy is well described [35]. In a series of 301 cases in North America identified over an 87 year period from medical records and published reports, the complication was seen in 12 per cent of patients and the large majority were of lymphoma and acute leukaemia [1]. Interestingly, primary lymphomas of extranodal sites including the brain and gastrointestinal tract were particularly frequent. The relative risk of malignancy was reported to be 100 times greater than that of the general population and increased with age. As yet, the influence of successful marrow transplantation and immune reconstitution on the risk of tumour development is unknown.

It is important to recognise that partially expressed forms of WAS exist and this diagnosis should be considered in all male infants with 'idiopathic' chronic thrombocytopenia [36–39]. In the majority of reported kindreds, the bleeding tendency is less severe than in the fully expressed form and life-threatening spontaneous haemorrhage is unusual. Generally, the immune deficiency is also less prominent and, although minor

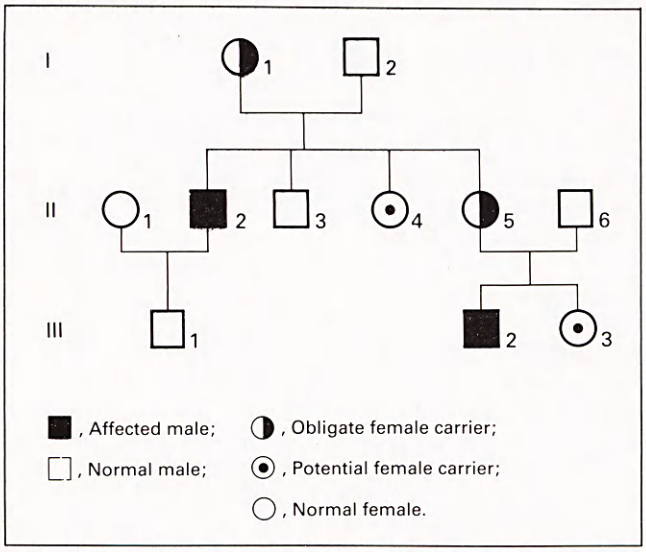

Fig. 1. *Pedigree of a family with an attenuated variant of Wiskott-Aldrich syndrome.*

abnormalities may be demonstrated *in vitro,* increased susceptibility to infection is commonly confined to splenectomised patients [36,37]. In one well characterised variant, for example, thrombocytopenia with small platelet size and a mild bleeding tendency is associated with elevated serum IgA, eczema and a nephropathy, but virtually intact immunity and normal life expectancy [39,40]. Part of a large kindred with this disorder identified in South Wales is shown in Fig. 1 and demonstrates the typical X-linked recessive inheritance pattern. The importance of correct diagnosis is clearly illustrated by a second family which has recently come to our attention in the Cardiff centre. Identical twin boys were diagnosed as suffering from familial 'immune' thrombocytopenia in 1964 at the age of seven years. Both were noted to have eczema, but there was no history of susceptibility to infection. Following a troublesome period of recurrent minor bleeding, one twin underwent a splenectomy which was complicated by pneumococcal septicaemia and meningitis ten days after the procedure. He recovered, but died three years later as the result of a rapidly fulminant Gram positive septicaemia. Recent investigations of the surviving twin clearly identified the disorder as an attenuated variant of WAS on the basis of small platelet size, low isoagglutinin titre and reduced serum IgM.

The eczema which is almost invariably present in the fully expressed disorder is also common in the attenuated variants and, indeed, may provide the first clue to the correct diagnosis in previously unrecognised cases. The lesions are indistinguishable from classical atopic eczema and the frequent association with positive prick tests, eosinophilia and elevated serum IgE suggests an allergic basis. Specific allergens should be identified and eliminated, but the course tends to be chronic and fluctuating. Topical steroids may be useful but secondary bacterial infection is common and special care is essential. Successful bone marrow transplantation frequently results in complete resolution.

Female carriers of WAS generally have normal platelet counts and platelet size and minor phenotypic abnormalities occasionally observed in these individuals are not sufficiently consistent to be applied to carrier detection. In particular, a biochemical test reported by Shapiro and colleagues [41] which involves differential inhibition of adrenaline-induced platelet aggregation by 2-deoxy-D-glucose has proved unreliable [25]. Studies by Gealy *et al.* [42] on a carrier heterozygous for A and B types of X-linked glucose-6-phosphate dehydrogenase showed that platelets and T lymphocytes from this female contained only one isoenzyme. It was concluded that, following normal random X inactivation during early embryogenesis, there is selection against the precursor cells in these series which carry the X chromosome with the WAS allele. However, there was no formal demonstration of selection in favour of cells expressing normal X chromosome products and neither could the possibility of extreme lyonisation in this individual be excluded. Second trimester prenatal diagnosis in male fetuses of carrier mothers at risk of having WAS can be achieved by fetal blood sampling and measuring platelet number and size. Holmberg and co-workers [43] have reported that there is no appreciable difference between these indices in mid-trimester fetuses, neonates and adults. By showing that an 18-week fetus of an obligate carrier mother had a normal platelet count and volume, they were able effectively to exclude WAS in the offspring. However, few would argue that there remains a need for earlier prenatal diagnosis using, if possible, more precise methodology.

Modern techniques of DNA analysis have provided a powerful new approach to the problems of carrier detection and prenatal diagnosis in inherited diseases. Although the precise location of the WAS gene defect has not been identified, close linkage has recently been

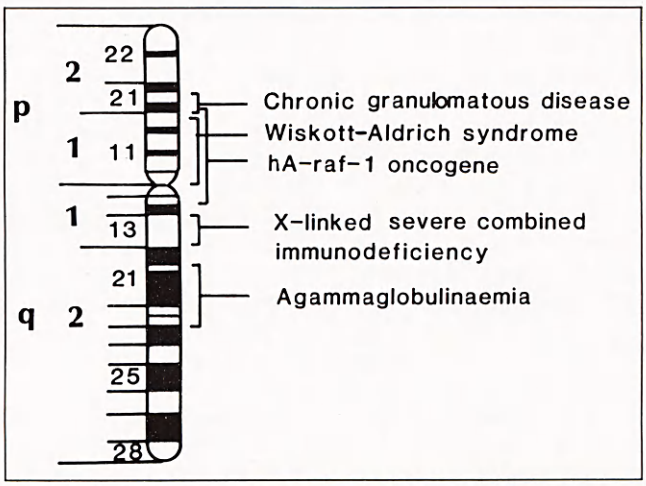

Fig. 2. *X chromosome showing approximate position of loci for the Wiskott-Aldrich syndrome, hA-raf-1 oncogene and other X-linked immune deficiency disorders. The murine X-linked immune deficiency (XID) locus maps to a region equivalent to q13→q22.*

reported between the WAS mutation in families with fully expressed disease and RFLP markers detected by two X chromosome specific DNA probes, 58.1 and L1.28 localised to the proximal short arm of the X chromosome [44] (Fig. 2). We have recently studied a large kindred with a non-lethal WAS variant and also demonstrated loose linkage with the L1.28 DNA marker (manuscript in preparation). This information should provide the first step towards accurate carrier detection using DNA analysis in families with WAS and, in conjunction with chorionic villus biopsy, a reliable means of first trimester prenatal diagnosis. In addition to providing practical benefit to afflicted families, DNA analysis and eventual gene localisation should answer more fundamental questions about the biology of WAS and, in particular, the nature and function of the normal gene product. More speculatively, although the increased risk of neoplasia in WAS is generally thought to be related to coexistent immune deficiency, altered expression of an oncogene in the vicinity of the WAS mutation might provide an alternative or additional mechanism of malignancy promotion. Intriguingly, the oncogene hA-raf-1 has recently been assigned to the proximal short arm/pericentric region of the X chromosome (Xp21-Xq11) by *in situ* hybridisation with most grains located at Xp11-Xp13 [45]). Molecular biology will clearly open an exciting new phase in our understanding of the pathogenesis of this devastating disorder.

Note added in proof

The view that sialophorin has an important role in lymphocyte function is strengthed by a recent report that the monoclonal antibody L10, which specifically interacts with this surface glycoprotein, stimulates T cell mitogenesis [46]. Furthermore, a rat lymphocyte sialoglycoprotein rich in O-linked disaccharide units has been recently cloned and sequenced [47]. Although the degree of structural homology with human sialophorin has yet to be determined, this development should nevertheless lead to a greater understanding of the biology of these surface molecules and their relevance to the functional defect in WAS.

Acknowledgements

I am grateful to Dr M. R. Haeney, Consultant Immunologist, University of Manchester School of Medicine, for helpful advice and to Mrs A. Porter for expertly typing the manuscript.

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