Disease mechanism: **Unravelling Wiskott–Aldrich syndrome**
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The gene responsible for Wiskott–Aldrich syndrome, a disease affecting platelets and lymphocytes, has been cloned and its protein product (WASp) found to interact with the GTPase Cdc42. WASp seems to provide a link between Cdc42 and the actin cytoskeleton, perhaps explaining the cellular defects underlying the disease.

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Jason P. was his parents' first son. He seemed normal at birth, but when he was three weeks old, his mother found bloody diarrhea in his diapers. Jason's pediatrician performed a blood count and found normal levels of all cells except platelets, so the bloody diarrhea was ascribed to faulty clotting resulting from a deficiency of platelets. Not only was the platelet count low, at 15 000 ml^{-1} (a normal level is more than 150 000 ml^{-1}), the platelets that were present were also unusually small. The pediatrician therefore diagnosed Wiskott–Aldrich syndrome (WAS), a disease characterized by defects in platelets and in immune function. A few months later, Jason developed an intensely itchy rash of eczema on his scalp, arms and legs. The eczema became infected with *Staphylococcus epidermitis* because Jason persistently scratched himself. Shortly thereafter Jason developed the first of several repeated middle ear infections, which was successfully treated with antibiotics. At age 3 he developed very severe and protracted chickenpox.
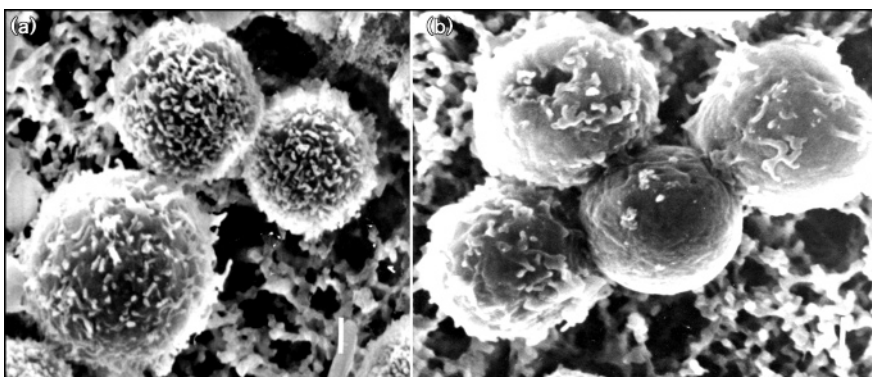
Fortunately for Jason, he and one of his older sisters expressed identical proteins of the major histocompatibility complex (MHC). It was therefore possible to transplant bone marrow from the healthy sister into Jason. Twenty days after the transplant, he exhibited complete bone marrow chimerism, and all his white blood cells had an XX karyotype, indicating that they developed from progenitors in his sister's bone marrow; he subsequently remained in good health. This case of Wiskott–Aldrich syndrome is typical: patients have severe defects in platelets and cells of the immune system (as manifested by susceptibility to infection and eczema) but other cells of the body are unaffected. The genetic defect underlying Wiskott–Aldrich syndrome is X-linked, and the gene has recently been identified [1].

Males with WAS fail to make an immune response to linear polysaccharide antigens [2,3] and respond poorly to protein antigens because of defective T-cell–B-cell interactions. Their lymphocytes are morphologically abnormal, in that they have a paucity of surface microvilli, assume bizarre shapes, and tend to be smaller than normal (Fig. 1) [4,5]. WAS platelets are also small: their average diameter is reduced by about 20 %. Curiously these abnormalities in cell volume can be largely corrected by surgical removal of the spleen [6], but it is unclear what perturbation in the WAS cells is being recognized by the spleen. What common denominator might explain how these two cell lineages — lymphocytes and platelets — are affected in Wiskott–Aldrich syndrome while other cell lineages are spared?

Three reports have been published recently which show that the product of the gene affected in WAS (Wiskott–Aldrich syndrome protein; WASp), interacts with the small GTP-binding protein Cdc42, a member of the Rho family of signal transducers. These observations

Figure 1

Scanning electron micrographs of (a) normal T cells, and (b) WAS T cells, revealing the differences in morphology described in the text.

arose from three different approaches. Aspentröm *et al.* [7], using a yeast two-hybrid protein–protein interaction screen with Cdc42 as 'bait', found a protein that binds to Cdc42 and which proved to be WASp. Symons *et al.* [8], using a gel-overlay technique with a probe of [γ-^{32}P]-labelled GTP complexed to Cdc42, found a labelled band of 62 kDa which likewise turned out to be WASp. We hypothesized that WASp might bind to a small GTP-binding protein involved in cytoskeletal organization, and so also found binding of Cdc42 to WASp [9]. All three groups found that WASp recognizes the GTP-bound and not the GDP-bound form of Cdc42, and that WASp reacts minimally or not at all with the Cdc42-related proteins Rac and Rho. These findings are exciting, because they connect WASp with a small GTP-binding protein that is known to regulate the organization of the cytoskeleton, and in particular the formation of filopodia and lamellipodia [10,11]. That the WASp–Cdc42 interaction does indeed have such a functional role is suggested by the observation in one of these three studies [8] that overexpression of WASp in transfected cells causes a clustering of polymerized actin that can be inhibited by simultaneous overexpression of a 'dominant-negative' mutant form of Cdc42.
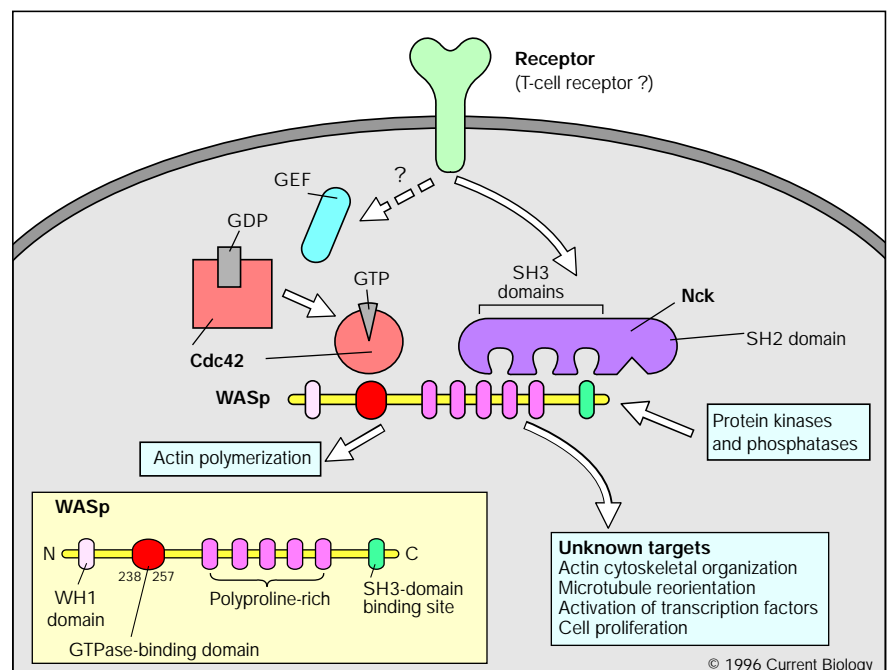
The recent observations allow us to begin to unravel the enigma posed by the phenotype of the WAS. The immune response in humans requires, for the most part, physical contact between T and B lymphocytes. Upon contact, the T cell polarizes its cytoskeleton toward the B cell [12,13], and this process seems to be regulated by Cdc42 [14]. In the absence of functional WASp, suggested to be a downstream effector of Cdc42, effective collaboration of T and B cells might therefore not occur, perhaps helping to explain the poverty of immune responses in WAS patients. It is also possible that WASp might regulate actin polymerization in other ways. For example, the carboxy-terminal portion of WASp contains polyproline stretches reminiscent of the proline-rich motifs found in VASP, a protein that regulates the polymerization of actin through interactions with the actin-binding protein profilin [15]. Furthermore, Symons *et al.* [8] found that the clustering of actin induced by overexpressing WASp is not observed with a truncated WASp missing the carboxy-terminal 59 amino acids. Whether or not WASp interacts directly with actin or through actin-binding proteins, in a way similar to VASP, is an area for further study that might illuminate the morphological abnormalities in WAS lymphocytes and platelets.

Other aspects of the phenotype of the disease remain enigmatic and are not readily reconciled with the discovery of WASp's binding to Cdc42. Circulating lymphocytes in people with WAS express surface molecules that are not expressed by resting cells and that immunologists take to indicate cell activation in response to stimuli (N. Gerwin, C. Friedrich, F.S.R and J.C. Gutierrez-Ramos, unpublished observations). Similarly, the platelets in their blood also seem to be constitutively activated [16]. So, it seems that WASp functions in a feedback loop that controls or down-regulates activation of these cell lineages. Indeed, attempts to stimulate T cells cultured from WAS patients fail to induce the up-regulated transcription of

Figure 2

A model for how the interaction of Cdc42 and WASp might be regulated by activated cell-surface receptors, leading to rearrangements of the cytoskeleton and modulation of transcription and cell proliferation.

interleukin-2 that is found in normal cells [17]. But, in contrast, signalling events such as calcium flux and phosphorylation of the T-cell receptor complex occur rapidly in WAS cells *in vitro*, just as in normal cells [17]. It therefore seems likely that WASp is positioned to exert its effects prior to the late events, such as transcriptional induction, but downstream of the early events, such as receptor phosphorylation (see Fig. 2).

There is no obvious explanation for the findings described above, but we might infer that WASp plays a role in at least two (or more) signaling pathways. In fact, the carboxy-terminal portion of WASp contains several sites predicted to bind Src homology 3 (SH3) domains, and the SH3-containing adaptor protein Nck seems also to be a WASp binding partner [18]. It is to be anticipated that yet other types of SH3-containing proteins, such as phosphatases and kinases, will also interact with WASp. We do not know the form in which WASp exists inside cells — whether it is a monomer or oligomerized, and whether or not it is phosphorylated. WASp may also change its intracellular localization in response to the physiological state of the cell, and any one of these modifications could influence its activity. Cell types other than platelets and lymphocytes do not seem to be affected by *WAS* mutation, so it is a logical possibility that a homologous and more ubiquitous protein(s) may lie at the crossroads between cytoskeleton organization and signal transduction cascades in other cell types.

Finally, there is a surprising effect of WASp. Like all X-linked genes, the *WAS* gene is expressed in women from only one of the two X chromosomes, which ordinarily leads to mosaicism. But heterozygous women who carry a *WAS* mutation exhibit preferential survival of bone marrow stem cells expressing the normal *WAS* gene [19]. Thus, all their blood cells have only wild-type WASp. This observation suggests a role for WASp in the interaction of bone marrow stem cells with stromal cells, which leads to stem-cell differentiation. Nonetheless, the stem cells in males with the same *WAS* mutation do survive, although their progeny lymphocytes are defective [19]. Clearly the discovery of the hitherto unknown WASp protein has provided a rich arena for many types of investigation of basic cell biology, and in particular the cell biology of the blood.

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