The Wiskott-Aldrich Syndrome: Studies of Lymphocytes, Granulocytes, and Platelets


Morphological, functional, and kinetic studies of lymphocytes, granulocytes, and platelets were carried out in three boys with classic and one with an "attenuated form" of the Wiskott-Aldrich syndrome (WAS). Lymphocyte counts, adequate during infancy, declined and were below normal by age 6. In vitro lymphocyte responses to irradiated allogeneic cells were reduced, but responses to mitogens with one exception, were normal. Antibody responses to bacteriophage OX 174 were uniformly abnormal: titers were low, immunologic memory was depressed, and the switch from IgM to IgG was impaired. The three boys with classic WAS did not respond to polysaccharide antigens. Neutrophils responded poorly to chemotactants and WAS sera generated less chemotactic activity than normal sera. A heat-stable inhibitor of thrombocytopenia, with platelets of half-normal size was found in all patients. Megakaryocyte cytoplasmic mass (a direct measure of marrow platelet production capacity) was normal, and small reductions in platelet survivals found (mean half-life, 5 days) were not sufficient to explain the degree of thrombocytopenia. Platelet turnover, which reflects the production of viable platelets, was 30% of expected, indicating that thrombocytopenia was due to ineffective thrombopoiesis. Similar measurements of platelets from mothers of our WAS patients were normal.

The Wiskott-Aldrich Syndrome (WAS) is a rare X-linked disorder. Affected boys present with bloody diarrhea, chronic eczema, recurrent bacterial and viral infections, thrombocytopenia, and an increased frequency of lymphoreticular malignancies. Studies of the immune system have suggested a defect of humoral immunity with specific inability to recognize polysaccharide antigens and a profound deficiency of cell-mediated immunity. The characteristic thrombocytopenia has been ascribed to a markedly reduced survival of defective platelets in which abnormalities of structure, function, and metabolism have been reported.

In this article we present comprehensive immunologic and hematologic studies of four unrelated patients with clinical and laboratory findings of WAS.

MATERIALS AND METHODS

Patients Studied

Each of the four patients was studied for at least 6 yr. In none was a positive family history obtained. One patient, AS, has a variant or "attenuated" form of WAS with less severe clinical manifestations.

EM, a 16-yr-old male reported previously, was recognized as having WAS at 18 mo. He has had recurrent bacterial and viral infections since infancy. At 1 yr of age he was hospitalized with chickenpox complicated by pneumonia, and at ages 2 and 9 he developed herpes zoster. At age 5 yr, a single platelet transfusion was required to stop an acute bleeding; no other blood products were given. Since age 6, the severity and frequency of his infections have decreased. On several occasions he developed nonpurulent arthritis involving mainly the large joints. Although his platelets are frequently below 20,000/µl, he has had no major bleeding episodes. He is smaller than his peers, bone age is slightly retarded, and puberty is delayed.

JW was 8 yr old when he died of overwhelming pneumococcal meningitis. Bloody diarrhea and abdominal distention were noted shortly after birth. A dry, scaly rash appeared at the age of 4 mo and progressed despite treatment with steroid cream. Certain foods seemed to worsen the skin eruptions. Bloody diarrhea occurred if he was exposed to wheat or cow's milk. Nose bleeds were frequent; platelet counts ranged from 6000 to 66,000/µl. Recurrent bacterial infections (pneumonia and otitis media) were also frequent and required antibiotic therapy. The diagnosis of WAS was made at the age of 1 yr. He never received blood products, except for modified gamma globulin. During the 2 yr prior to his death he had extensive molluscum contagiosum of the scalp, face, neck, trunk, and extremities.

CR is a 7-yr-old boy who presented with persistent diarrhea during infancy. At 1 yr of age he developed petechiae, and the diagnosis of WAS was made. Subsequently, he had numerous episodes of spontaneous epistaxis requiring emergency hospitalizations and transfusions of whole blood and packed red cells. His platelet count was frequently below 20,000/µl (range 5000-73,000/µl). He had no major infections during the first year of life, but later developed recurrent otitis media and several episodes of pneumonia. Recently, he has had recurrent herpes simplex infections.

AS is an 11-yr-old boy with a variant form of WAS. At the age of 3 mo he presented with oral candidiasis and a diaper rash, which remained until nine mo of age. No bacterial infections were reported.

From the Howard Hughes Medical Institute; Division of Arthritis and Immunology, Department of Pediatrics, Division of Hematology, Department of Medicine, and the Department of Laboratory Medicine, University of Washington School of Medicine, Seattle, Wash.; the Puget Sound Blood Center, Seattle Wash.; and the Evans Memorial Department of Clinical Research, Boston University Medical Center, Boston, Mass.

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Address reprint requests to Dr. Hans D. Ochs, Department of Pediatrics RD-20, University of Washington School of Medicine, Seattle, Wash., 98195.

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until 6 yr of age. When 6 mo old, the patient developed petechiae, episodes of epistaxis, and a low platelet count was noted (5000–50,000/μl). Immune-mediated thrombocytopenia was considered, and he was treated intermittently with steroids without improvement. Following splenectomy at age 5, his bleeding tendency disappeared, although his platelet counts were still low, ranging between 50,000 and 150,000/μl. One year after splenectomy, he developed pneumococcal meningitis and subsequently has had several episodes of pneumococcal septicemia and one bout of H. influenza meningitis. He has not received any blood products.

Lymphocytes
Surface immunoglobulin (SIg) bearing lymphocytes were identified by a modification of Papamichail's method.11 Peripheral blood lymphocytes (PBL) separated on Ficoll-Hypaque gradients were incubated with fluorescein-labeled heavy-chain-specific antisera to IgG, IgA, IgM (Wellcome Reagents Ltd., Beckenham, England) and IgD (Behring Diagnostic, Sommerville, N.J.), and an anti-total-immunoglobulin antisera (anti-kappa and anti-lambda, Fab', fragment, Cappel Laboratories, Cochranville, Pa). The percentage of labeled cells was determined with an epi-illuminated fluorescence microscope (Zeiss IV FL epifluorescence condenser).

The percentages of circulating lymphocytes with sheep red blood cell (SRBC) receptors (E rosettes) or with C3b receptors (EAC cells/well) were cultured in RPMI (RPMI 1640, Grand Island Biological Co., Grand Island, N.Y.) with 10% fetal calf serum (FCS, Grand Island Biological Co.) in a microculture system in the presence of varying doses of phytohemagglutinin (PHA, Burroughs Wellcome, Research Triangle Park, N.C.) concanavalin A (Con-A, Cal Biochem, San Diego, Calif.), and pokeweed mitogen (PWM, Grand Island Biological Co.). Mixed leukocyte cultures (MLC) (total volume 0.2 ml RPMI, 10% FCS) contained 10^5 responding cells and 10^5 irradiated (I 500 rad) lymphocytes from a normal, unrelated adult control as the stimulator cell. Eighteen to 24 hr before harvesting, cultures were pulsed with 2 μCi ^3H-thymidine (3H-TdR), specific activity 6.7 Ci/mM (New England Nuclear, Boston, Mass.). Cultures were terminated on day 3 for PHA, day 5 (in some instances on day 4) for Con-A and PWM stimulated cultures, and on day 6 or 7 for the MLC. Cells were collected with a multichanneled cell harvester on glass-fiber filters, and radioactivity was counted with a liquid scintillation counter.

Delayed Hypersensitivity Skin Tests
The following skin tests were applied: Dermatophytont O (Hollister-Stier) diluted 1:100; Streptokinase-Streptodornase (SK/SD, Lederle), 40 U SK, 10 U SD per 0.1 ml; Trichophyton (Hollister-Stier) diluted 1:30; PPD 5 U/0.1 ml; and Mumps skin test antigen (Eli Lilly).

Serum Immunoglobulins
Concentrations of the serum immunoglobulins IgG, IgA, and IgM were determined by single radial immunodiffusion using commercially available plates (Hyland, Los Angeles, Calif.). IgD and IgE were measured by radioimmunoassay in the laboratory of Dr. Douglas Heiner, Harbor General Hospital, Torrance, Calif.

Antibody Responses
Bacteriophage OX 174 was grown in Escherichia coli, harvested, purified, and sterilized as previously described.17 The final preparation of phage contained × 10^{12} plaque-forming units (PFU)/ml. After informed consent was obtained, phage was given intravenously (i.v.) at a standard dose of 2 × 10^9 PFU/kg body weight. Five to six weeks after the primary immunization, a second dose was injected intravenously. The third dose of phage was given subcutaneously (s.c.) several months later. Two years later, three patients received a fourth immunization (i.v.). In addition to 23 normal controls who received 2 phage immunizations intravenously, 3 normal controls were immunized exactly as the WAS patients. After each phage injection, serum was collected at weekly intervals for antibody determination. Antibody activity was determined by phage neutralization and expressed as the rate of phage inactivation or K value (Kv), derived from a standard formula.17 Antibody susceptibility to reducing agents was determined by the method of Grubb and Swahn, using 2-mercaptoethanol.18 The antibody titers (Kv) conform to lognormal frequency distributions. Hence logarithms of K values are used to compare results between groups, and the data are expressed as geometric means.

When 6 yr or older, the patients were immunized subcutaneously with tridecavalent pneumococcal polysaccharide (PPS) vaccine (Lederle Laboratories, Pearl River, N.Y.) containing 50 μg of the following purified pneumococcal capsular polysaccharides—types 1, 3, 4, 6A, 6B, 7, 8, 9, 12, 14, 18, 19, and 23; no side effects were observed. Blood, collected 3 wk later, was assayed for type-specific capsular antibodies to the 13 types, except type 6B (which cross-reacts with 6A), by Dr. Gerald Schiffman, Downstate Medical Center, Brooklyn, N.Y.19 Twenty-seven normal children similarly immunized served as controls.

Neutrophil Chemotaxis
Neutrophil chemotaxis was studied by a previously reported method,20 employing 51Cr-labeled neutrophils and a two-filter Boyden chamber system with partially purified human C5a and endotoxin-activated human serum as chemoattractants.21 During the labeling procedure, the cells were washed 3 times to remove unbound Cr. Except for kinetic experiments, a standard 3-hr response was determined. Each test was run in quadruplicate, with correction for the specific activity of each of the cell preparations. Chemotaxis was expressed as percentage of the simultaneously studied normal control neutrophils. In addition, the chemotactic activity of patient serum, either alone or activated by endotoxin was compared to that of normal serum using normal test neutrophils. The chemotactic inhibitory activity in patient serum was evaluated by determining the chemotactic response to C5a of normal neutrophils suspended in 10% patient serum compared to the response of the same cells in 10% normal serum.22

Platelet Megakaryocyte System
Platelet counts were estimated on blood collected in EDTA, and Coulter size distributions were evaluated on both EDTA and citrated blood.23,24 The sizing system was modified for hydrodynamic focusing to enhance resolution.23,24 Distributions were recorded using logarithmic pulse amplification to simplify computer analysis,23,24 and the system was calibrated with latex particles to give appropriate volumes for EDTA-sphered platelets.23 The shape difference between the EDTA and citrate platelets was measured by the EDTA/citrate ratio of the two mean platelet volumes (MPV).23-27 MPVs were estimated from the geometric mean, and geometric standard deviation of the computer fitted lognormal distribution.23,24,27,28 The thrombocrit, computed from platelet count and MPV, indicates the percentage of blood volume represented by platelets.

Platelet survival was determined from the disappearance of radioactivity from blood samples after injection of autologous and,
LYMPHOCYTES, GRANULOCYTES, PLATELETS IN WAS

in two patients, allogeneic \(^{35}^{35} \text{Cr}\)-labeled platelets \(^{23,29}\) After labeling, a final spin of the platelet concentrate was done in two sterile 15-ml test tubes at 200 g for 5 min to remove leukocytes and erythrocytes. The final injected platelet preparation contains less than 1% of contaminating cells. Platelet survival time was estimated using a computer-fitted gamma function. \(^{30,32}\) and platelet recovery was calculated as previously described. \(^{28}\) Platelet mass turnover was determined by multiplying the standard platelet turnover measurement by the MPV.

Bleeding time was measured by a standardized template method. \(^{33}\) Platelet aggregation was estimated from changes in optical transmission after the addition of adenosine diphosphate (ADP), epinephrine, and collagen \(^{34}\) to those blood samples with platelet counts high enough to achieve the standard 300,000/\(\mu\)l platelet count in the platelet-rich plasma so that reliable measurements could be made.

Platelet-bound IgG antiplatelet antibody was determined using \(^{125}\)I-labeled rabbit anti-human IgG antibody. One milliliter of platelet-rich plasma (PRP) containing \(\times 10^7\) platelets is added to a Sepharose 2B column, which removes nonspecifically bound gamma globulin. \(^{35}\) Then, \(\times 10^9\) of the recovered platelets are mixed with a purified rabbit \(^{125}\)I-labeled anti-human IgG for 45 min at room temperature. Platelet-bound anti-human IgG was separated from unbound antibody by passing the mixture over a second Sepharose 2B column. Utilizing normal PRP \((n = 30)\) less than 5% of the anti-human IgG added remains attached to the platelets. The test is considered positive when the patients’ platelets show an amount of anti-human IgG bound that is at least twice the control. The test reflects directly the amount of antibody attached to the platelets. \(^{36}\)

Marrow biopsy cores were obtained and prepared for light microscopy as previously described. \(^{37}\) Megakaryocyte number, diameter, and volume were measured, and total marrow megakaryocyte cytoplasmic mass was calculated as previously outlined. \(^{21,37,38}\) The results were compared with data obtained in 13 normal subjects.

Statistics

Unless otherwise indicated, results are given as the mean \(\pm\) 1 standard deviation, and the Student’s t test was used to compare population means.

RESULTS

Lymphocytes

Peripheral blood lymphocyte (PBL) counts determined serially during the period of observation are shown in Fig. 1. While lymphocyte counts were normal in all four patients during the first few years of life, a consistent decrease in the absolute number of lymphocytes was found later. By age 6, lymphocyte counts were below the normal range in the 3 patients with the classic form of WAS. AS, with the variant form, shows a similar trend but his lymphocyte counts remain within the normal range.

The distribution of T and B cells in the peripheral blood are shown in Table 1. Three patients had normal or low-normal percentages of E-rosette-forming cells; one patient (EM) had a significantly decreased percentage. However, due to the low counts of peripheral blood lymphocytes, all patients had decreased absolute numbers of peripheral blood T lymphocytes. C3b-receptor-bearing lymphocytes (EAC rosettes) and surface Ig-positive lymphocytes were within normal limits (Table 1). A high proportion of PBL bearing no demonstrable surface markers was noted in three patients.

Lymphocytes from one patient (JW) were consistently unresponsive to in vitro stimulation with PHA, Con-A, and PWM. Lymphocyte responses to optimal doses of mitogens were normal or only moderately depressed in the other three patients. On some occasions, we observed depressed lymphocyte responses if suboptimal doses of mitogens were used, similar to the findings reported by Oppenheim et al., \(^{41}\) however this abnormality was inconsistent and observed in only two patients (EM, CR). Stimulation of Wiskott-Aldrich lymphocytes with irradiated allogeneic cells (MLC) showed significantly depressed \(^3\)H-thymidine incorporation in all four patients (Fig. 2).

Serum Immunoglobulins (Table 1)

The concentration of serum IgG was below the mean but within the range of age-matched controls. \(^{42}\) During the time of observation (4–7 yr) serum IgG levels did not change significantly. The IgA concentrations were generally higher than or in the upper range of normal. With one exception (JW), serum IgM was lower than reported for age-matched controls. \(^{42}\) Serum IgE levels were elevated in two patients; the concentration of serum IgD was normal. \(^{43}\)
Antibody Responses

After i.v. administration of bacteriophage ØX 174, the antigen disappeared from the circulation in both normals and patients within 1 wk. In contrast, antibody responses to the initial and subsequent injections of phage were clearly different between controls and patients (Fig. 3). In normal subjects, the peak antibody titer is reached 2 wk following the first phage injection; after a second i.v. phage injection, titers rise briskly to high levels, indicating amplification and memory; recall responses consist predominantly of IgG antibody; a third immunization, given subcutaneously, causes brisk antibody responses and continuous rises in titer during the following 4 wk. In WAS patients the primary antibody response peaked at 1 wk then declined rapidly; no antibody could be detected at 4 wk. A second i.v. injection of phage resulted in a low and rapidly declining antibody titer of IgM, similar to the primary response. Four weeks following the second injection, antibody could be demonstrated in only one of the four patients (AS). A third dose of phage, given s.c. initiated a quantitatively and qualitatively almost normal antibody response in the variant WAS patient (AS); the other three patients responded with a depressed antibody titer, consisting of IgM only. The
titer declined rapidly in all four patients. Three patients were immunized a fourth time intravenously. In two patients antibody titers were low but predominantly IgG. The antibody titer of the third patient (EM) was low, decreased rapidly, and consisted of IgM only.

Antibody titers to 12 type-specific pneumococcal polysaccharides (PPS) were determined before and after immunization with a tridecavalent vaccine and are shown in Table 2. The amount of antibody detected in the WAS patients before vaccination was lower than in the 27 control children. Three patients failed to respond to PPS. The patient with the variant syndrome (AS) showed a significant antibody rise to 3 of the 12 types. In contrast, normal children responded well to all types of PPS with the exception of type 19.

**Neutrophil Chemotaxis**

The chemotactic responses of extensively washed WAS and control neutrophils to C5a and to endotoxin-activated sera are shown in Table 3. With C5a as the attractant and with endotoxin-activated WAS (but not normal) serum, we could clearly demonstrate a defect of the WAS neutrophils. This defect was most prominent at short incubation times, as has been observed in other patients with abnormal chemotaxis. A possible defect of WAS serum was further investigated using normal neutrophils (Table 4). Chemotactic activity of nonactivated WAS sera was

**Fig. 3.** Antibody responses to four injections of bacteriophage ØX 174 given intravenously (i.v.) or subcutaneously (s.c.). (O—O) represents the mean titer and the shaded area the range of normal controls. Antibody responses of the WAS patients are indicated individually. Controls produce predominantly IgG antibodies during the second, third, and fourth immunization. In contrast, only AS (during third and fourth immunization) and CR (fourth) made IgG antibody to phage.

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*Antibody response expressed as nanograms of antibody nitrogen per milliliter.
†Pre- or postimmunization.
‡Geometric means of 27 normal children.
activated serum as attractants.

Incubation periods of 1 or 2, or 3 hr with C5a and 3 hr with endotoxin-activated serum were used to examine with serum from JW since his neutrophils were hyperactive and for each patient at different times. Platelet counts ± were reduced by 62% of normal serum. The inhibitor of JW's serum was reduced in all four and that of endotoxin-activated serum in three of the patients. These findings suggested the possibility of a serum inhibitor of chemotaxis. This inhibition was not due to reversal of the chemotactic gradient by a chemotactic factor in the patient serum, since it had less chemotactic activity than normal serum (Table 4).

Table 3. Chemotactic Response of WAS Neutrophils to Attractants

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*Chemotaxis is expressed as percent of normal control neutrophils for incubation periods of 1, 2, or 3 hr with C5a and 3 hr with endotoxin-activated serum as attractants.

† p < 0.01 versus normal control neutrophils.

Platelet Megakaryocyte System

Platelet counts varied greatly both within the group and for each patient at different times. Platelet counts ranged from 5000 to 153,000/μl, with the highest counts in the splenectomized patient, AS, who had the variant form of WAS (Table 5).

Over the course of 5 yr, the platelet size of each patient was studied at least three times and those of many relatives more than once. The three patients with typical WAS all had marked thrombocytopenia, mean platelet volumes (MPV) about half those of control subjects, and therefore low thrombocrits of approximately 0.01% compared with a normal range of 0.14%–0.31% (Table 5). The splenectomized WAS-variant (AS) also had small platelets, but only a moderate thrombocytopenia and a thrombocrit of 0.06%. Platelet counts and MPV of the available relatives tested, including the mothers, were all normal. While the platelets of all subjects exhibited great variability in spreading and shrinking on blood films, the frequency distributions of the electric volume measurements were typically lognormal. The EDTA platelets were spherical and the citrate platelets were discoid, resulting in normal EDTA/citrate ratios (1.15–1.40). During and after periods of infection, platelet counts increased as much as four- to fivefold, but the MPV or the EDTA platelets remained consistently reduced throughout these fluctuations.

Autologous and allogeneic recoveries were normal in these patients, averaging 65%. In one patient, CR, autologous recovery was 33% and allogeneic 54%. This patient had a palpably enlarged spleen, which could account for the reduced recoveries. Autologous platelet survivals were moderately shortened to about half-normal, i.e., 5.0 days ± 1.3 compared with 9.5 days ± 0.6 days in normal subjects (p < 0.001) (Table 5). Allogeneic platelet survival, determined in two patients, was comparably shortened to 4.8 days in patient EM (autologous 5.3 days) and 3.8 days in CR (autologous 4.2 days). Calculations of platelet mass turnover demonstrated a significant platelet production defect in these patients, i.e., 0.9 × 10^4 fl/μl/day ± 0.4 compared with 3.0 × 10^5 fl/μl/day ± 0.3 in normal subjects. In contrast, marrow megakaryocyte mass was normal in two subjects and significantly increased in one, indicating disparity between “marrow substrate” and circulating product (Table 6), i.e., ineffective thrombopoiesis. Platelet kinetics in the four mothers revealed no abnormalities in count, recovery, survival, or turnover, except in the mother of EM whose platelet survival was about half-normal at 5.7 days (Table 5).

The amount of anti-human IgG bound to the patient’s platelets was between 2.7% and 5% of the amount added—well within the normal range.

Platelet Function

Bleeding time measurements were greatly prolonged (Table 5). The values were longer than predicted for the degree of thrombocytopenia, and thus the results may reflect both the marked thrombocytopenia and the reduced circulating platelet mass as measured by the thrombocrit. AS was the only patient with a platelet count high enough to perform reliable
aggregation studies, and they gave normal results. Tests of platelet function in the mothers, including bleeding time and aggregation to ADP, collagen, and thrombin, were all within normal limits.

**DISCUSSION**

For several years we have evaluated immunologic and hematologic aspects of four patients with WAS. One of the four patients developed infections only after splenectomy and was immunologically less impaired, but showed many of the platelet, lymphocyte, and granulocyte abnormalities typical for the WAS; he resembles patients described as “form fruste” or “attenuated form” of the WAS.47 52 The other three patients were typical of the syndrome.

The immunologic defects of all four patients were remarkably similar and confirm the observations reported by others.4 5 41 53 Isohemagglutinin titers were extremely low and serum IgM was below the normal range42 in three individuals. Serum IgA and IgE levels were moderately elevated when compared with age-matched controls.42 43 The immunologic response to pneumococcal polysaccharides was markedly depressed. More informative was the extremely low antibody response to the T-cell-dependent antigen, bacteriophage $\Phi X$ 174. The antibody response following a second injection of bacteriophage was identical to the primary response, indicating lack of immunologic memory and inability to switch from IgM to IgG antibody production. The most widely accepted theory for the humoral immune defect of WAS is abnormal recognition and processing of polysaccharide antigens.45 The fact that immunization with the protein-coated DNA phage results in a markedly abnormal immune response suggests that the recognition defect theory has to include protein antigens. Additionally, the similarity of the immune response of the WAS to those we have observed in patients with selective T-cell deficiencies, including the diGeorge syndrome, and in nude mice,54 suggests that the humoral immune defect may reflect an underlying T-cell abnormality, possibly a lack of T-helper cells. In our patients, peripheral blood lymphocyte counts—normal during infancy—showed a progressive decline, resulting in lymphopenia by age 6 in three patients, at which time the absolute number of T lymphocytes was dramatically decreased, while B cells of all immunoglobulin classes tested were present in normal numbers. Furthermore, a partial T-cell defect is suggested by the increased incidence of recurrent viral infections, absent delayed hypersensitivity skin tests, depressed lymphocyte responses to allogeneic cells, and by possible in vivo benefit from transfer factor,55 the demonstration of an in vitro effect of thymosin$^{56}$ and thymus epithelial cells upon T-cell maturation.57 Thus, many of the immunologic abnormalities might be explained by a selective T-cell defect. The lack of antibody responses to pneumococ-
cal polysaccharides (generally considered to be T-cell independent) may indicate that WAS patients have in addition a B-cell defect, or that their cells are unable to recognize and process polysaccharide antigens.45

The chemotactic responses of extensively washed WAS neutrophils showed a significant cellular defect that was most pronounced if the incubation time was short. This has been observed in other patients with abnormal chemotaxis.44,45 In addition, WAS serum, nonactivated or activated, had decreased chemotactic activity for normal granulocytes, contained a heat-stable inhibitor of granulocyte chemotaxis, and had elevated concentrations of IgE. WAS thus represents another example of the association between recurrent infections, hyperimmunoglobulin E, and defective neutrophil chemotaxis.45,58-61 The explanation is not known although an adverse effect of IgE-mediated histamine release has been suggested.58 A serum chemotactic inhibitor has not generally been detected in the hyper-IgE patients. However, the defect in monocyte chemotaxis in WAS patients described by Altman et al. was associated with a humoral chemotactic inhibitor, possibly representing a lymphocyte-derived chemotactic factor that might deactivate circulating monocytes or neutrophils.62 Our data do not exclude the possibility of inhibition by an antineutrophil antibody.

The characteristic finding of thrombocytopenia with reduced platelet volume7,9-11 was present in all patients. We found autologous platelet survivals of approximately half-normal (survival times of 5.0 days ± 1.3, 1 SD) in contrast to the marked shortening (2 days) reported by others.9 In addition, immediate posttransfusion platelet increments (i.e., recovery) were normal in our patients (64%-87%, mean 76%), except for one patient (CR) who had hypersplenism (recovery of 33%).46 This is compared to significant reductions (recoveries of 8%-30%) in previously reported studies.63,64 Infusion of allogeneic platelets gave similar recoveries and survivals. Normal allogeneic survivals have been found by others.7,8,10 Thus, the slightly shortened platelet survivals we found appear related to factors extrinsic to the platelets, not to a significant intrinsic platelet defect adversely affecting platelet recovery and survival as previously suggested.9,11 In fact, studies of either autologous or allogeneic platelet survivals in nontransfused patients with thrombocytopenia secondary to marrow failure have consistently demonstrated modest reductions in platelet survivals very similar to those found in the WAS patients.46 Thus, abnormal platelet survivals may reflect a factor common to all thrombocytopenic patients, not WAS alone. In addition, we found no evidence of immunologically mediated platelet destruction in WAS, confirming the observations of Gröttum et al.9

The most striking abnormality was the calculated platelet turnover, which was approximately 30% of the value found in normal subjects. The discrepancy between a normal to increased marrow substrate available for platelet production (megakaryocyte cytoplasmic mass) and the low rate at which platelets actually appeared in the circulation (platelet turnover) characterizes the platelet production defect known as ineffective thrombocytopoiesis.23,39,40 This abnormality may be due either to a defect in platelet demarcation, resulting in failure of platelet release from megakaryocytes in the bone marrow, or to intramedullary platelet destruction.

As Gröttum noted,9 bleeding times were prolonged. The degree of prolongation was greater than the reduction in platelet count. The decrease in absolute platelet mass secondary to the reduced platelet volume may, as previously published,13 explain the discrepancy. Platelet aggregation studies, performed only in patient AS (the other individuals had inadequate platelet counts), were normal. Decreased responsiveness to collagen and ADP,9,12 decreased platelet adhesiveness,9 a lack of response to epinephrine and polystyrene latex particles12 have been interpreted as platelet functional defects in some patients, but the severe thrombocytopenia in these patients compromises interpretation. Platelets from WAS patients have also been reported to have decreased ADP and ATP levels as well as defects in glycolysis and phosphorylation.10 Unfortunately, these metabolic abnormalities were related to numbers of platelets not platelet volume, and the reductions may reflect nothing more than the known decreased volume of WAS platelets. Abnormal platelet aggregation has been observed in patients with severe combined immunodeficiency and adenosine deaminase deficiency.65-67 These infants, however, show no bleeding tendency, and the number and size of their platelets are normal. Furthermore, patients with WAS have normal levels of red blood cell adenosine deaminase.68 Ultrastructurally, WAS platelets were reported to be normal11 or lacking granules and mitochondria.9,10 Some of these abnormal findings of function and structure might be due to platelet damage encountered during platelet preparation in these severely thrombocytopenic patients.

The mothers of our patients had normal platelet numbers, size, function, and, except in one, survival times. The survival time of approximately half-normal (5.7 days) found in only one of the mothers cannot be given any special significance since it was an isolated finding. In contrast, Baldini,10 who studied two moth-
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ers of patients with WAS, found a defective secondary wave of platelet aggregation induced by epinephrine. Grøttum noted that one of three mothers of his patients with WAS had low platelet counts but normal platelet function.9 Recently, Shapiro et al. reported that adrenalin-induced platelet aggregation was inhibited by 2-deoxy-D-glucose, an inhibitor of glycolysis, in WAS carriers but not in normal female controls.90 This observation has yet to be confirmed.

This study has demonstrated diverse abnormalities of lymphocytes (both B and T cells), granulocytes, and platelets in the WAS. Altman has, in addition, reported defective monocyte function.62 Thus, of the progeny of the hematopoietic stem cell, it appears likely that all but the erythrocytes are effected. The disease possibly reflects a basic abnormality within the stem cell itself, rendering the differentiated progeny defective in some undetermined fashion. A unifying concept to explain these abnormalities is not yet apparent. Further studies are required to identify the abnormal or absent gene product in this hereditary disease.

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REFERENCES

14. Mendes NF, Tolnai M, Silveira NPA, Girldtsen RB, Metzgar RS: Technical aspects of the rosette tests used to detect human complement receptor (B) and sheep erythrocyte-binding (T) lymphocytes. J Immunol 111:860, 1973
60. Pincus SH, Thomas IT, Clark RA, Ochs HD: Defective neutrophil chemotaxis with variant ichthyosis, hyperimmunoglobulinemia E and recurrent infections. J Pediatr 87:908, 1975
63. Murphy S: Personal communication
The Wiskott-Aldrich syndrome: studies of lymphocytes, granulocytes, and platelets

HD Ochs, SJ Slichter, LA Harker, WE Von Behrens, RA Clark and RJ Wedgwood