T(H1- and T(H17)-induced inflammation, inhibition of nuclear factor kappa b, and upregulation of IL-10.9,10 As such, PPARγ agonists are being trialed for a number of inflammatory diseases (www.clinicaltrials.gov). We have shown that PPARγ activation occurs downstream of the activated NADPH oxidase and is lacking in CGD phagocytes, but can be restored by pioglitazone.2,7 PPARγ agonism restores effectorcytosis by macrophages in murine CGD, and, as shown here, in human CGD monocytes as well. This was demonstrated after both ex vivo treatment of human CGD monocytes and treatment of 2 patients with CGD with pioglitazone. As in our earlier studies, PPARγ agonism was also accompanied by enhanced phagocyte mitochondrial ROS production.7 Whether mitochondrial ROS contributes to effectorcytosis is an area for future study. Whether pioglitazone will decrease inflammation in CGD and whether pioglitazone-enhanced mitochondrial ROS will bolster host defense in human CGD, as it did in murine models,1 are key unanswered questions. If human data are similar to preclinical models, pioglitazone, an on-the-shelf therapy, may ameliorate both immunodeficiency and inflammatory aspects of CGD.

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LETTERS TO THE EDITOR

Broad spectrum of autoantibodies in patients with Wiskott-Aldrich syndrome and X-linked thrombocytopenia

To the Editor:

Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are allelic diseases caused by mutations of the WAS gene.3 Autoimmune manifestations (especially cytopenias, inflammatory bowel disease, vasculitis, arthritis, and IgA nephropathy) affect between 24% and 72% of patients with WAS in various series, with important implications on quality of life and survival.2 Although patients with XLT do not have autoimmune manifestations at diagnosis, some of them can have autoimmunity over time.3

To investigate in greater detail and compare the degree of immune dysregulation in WAS and XLT, we have studied 17 patients with WAS and 10 patients with XLT. The clinical and laboratory features of the patients are reported in Table I.

Plasma samples from patients with WAS/XLT were diluted 1:100 in PBS and 100 μL of the dilution was incubated in duplicate with an autoantigen proteomic array (University of Texas Southwestern Medical Center, Genomic and Microarray Core Facility), which includes 67 and 77 self-antigens, respectively, to analyze the frequency, antigen specificity, and isotype composition of autoantibodies. Plasma from 6 healthy control subjects and 5 patients with systemic lupus erythematosus served as negative and positive controls, respectively. The arrays were then incubated with Cy3-labeled anti-human IgG and Cy5-labeled anti-human IgA antibodies, respectively, to define the IgG or IgA isotype specificity of the autoantibodies. Tiff images were generated by using the GenePix 4000B scanner (Molecular Devices, Sunnyvale, Calif) with laser wavelengths of 532 nm (for Cy3) and 635 nm (for Cy5) and analyzed with GenePix Pro 6.0 software. Net fluorescence intensity (defined as the spot minus background fluorescence intensity) data obtained from duplicate spots were averaged. Data were normalized as follows. Across all samples, the immunoglobulin-positive controls (IgG or IgA) were averaged, and the positive controls in each sample were divided by the averaged positive control, generating a normalization factor for each sample. Each signal was then multiplied by the normalization factor for each block (sample). For each antigen, values from healthy donor samples (n ≥3) were averaged. For each sample, ratios were then calculated between the value in the sample and the average of values in healthy donors plus 2 SDs, thus defining relative autoantibody reactivity (RAR) of the sample. RAR values of greater than 1 were considered positive. A heat map of the ratio values was generated by using MultiExperiment Viewer software (DFCI, Boston, Mass). Significant differences in autoantibody signal between groups were assessed by using Significance
Patients with autoantibody multireactivity had significantly higher serum IgA levels compared with patients with reactivity to less than 20% of the self-antigens tested, and a similar trend was observed for serum IgG levels (see Fig E2 in this article’s Online Repository at www.jacionline.org). Self-antigens to which autoantibodies were demonstrated in more than 20% of patients with WAS/XLT were defined as “common autoantigens.” The 25 most common IgG and IgA autoantibodies are reported in Fig E3 in this article’s Online Repository at www.jacionline.org. Of note, 9 (36%) of the 25 top most common autoantigens were the target of both IgG and IgA autoantibodies (mitochondrial antigen, fibrinogen IV, entactin, M2 antigen, myosin, elastin, and fibrinogen IV). Of note, 9 (36%) of the 25 top most common autoantigens were the target of both IgG and IgA autoantibodies (mitochondrial antigen, fibrinogen IV, entactin, M2 antigen, myosin, elastin, and fibrinogen IV).

Multiplex immunologic abnormalities have been identified that might account for immune dysregulation in patients with WAS, including impaired function of regulatory T and regulatory B cells, defective apoptosis, abnormalities of the distribution and diversity of T and B lymphocytes, and defective function of T and natural killer cells, resulting in impaired clearance of autoantibodies.
pathogens and persistent inflammation. Moreover, Wiskott-Aldrich syndrome protein (WASP)–deficient plasmacytoid dendritic cells are hyperresponsive to Toll-like receptor 9 stimulation and produce high amounts of type 1 interferon, which might also contribute to autoimmunity. More recently, we and others have identified B-cell autonomous effects of WASP deficiency that are likely to play a critical role in the autoimmunity of the disease. These include (1) hyperresponsiveness of WASP-deficient B cells to stimulation through the B-cell receptor and Toll-like receptors; (2) accumulation of B lymphocytes with a characteristic phenotype (CD21lowCD38low), which is indicative of a type 1 interferon signature and a marker of self-reactivity; (3) preferential use of immunoglobulin variable genes that are enriched in patients with autoimmune disease and decreased somatic hypermutation; (4) increased release of immature B cells from the bone marrow to the periphery; (5) increased levels of B cell–activating factor of the TNF family serum; and (6) decreased regulatory B-cell function. In our series increased levels of B cell–activating factor of the TNF family serum were found not only in patients with WAS but also in those with XLT (Table I).

To our knowledge, our study represents the first attempt at extensively analyzing the frequency and diversity of autoantibodies in patients with WAS versus those with XLT. Our data indicate that biological signs of immune dysregulation are a characteristic feature of patients with loss-of-function mutations of the WAS gene, irrespective of the severity of the clinical phenotype. This biological signature of immune dysregulation might set the stage for progressive development of clinical manifestations of autoimmunity also in patients with XLT. Consistent with this, 3 of the patients with XLT included in this study (XLT 18, XLT 19, and XLT 33) had cutaneous vasculitis later in the course of their disease, and 1 of them (XLT 18) also had arthritis, a pattern that has been reported in several other patients with bona fide XLT. These data strongly suggest that XLT should not be considered a distinct disease entity but rather part of the clinical spectrum of WAS. Prospective longitudinal studies are needed to assess whether differences in the amount, diversity, and avidity of autoantibodies produced are predictive of development of clinical manifestations of autoimmunity in patients with XLT/WAS.

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Impaired microbial killing by neutrophils from patients with protein kinase C delta deficiency

To the Editor:

Neutrophils are of critical importance in the host defense against bacterial and fungal infections. Upon microbial phagocytosis, neutrophils use at least 2 major and well-established intracellular killing mechanisms, that is, the production of reactive oxygen species (ROS) by the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, and the release of antimicrobial components from intracellular granules.1 Protein kinase C delta (PKCδ) is a serine and threonine kinase and a member of the so-called novel PKC isoenzyme family.2 PKCδ is expressed in neutrophils3 and can mediate the phosphorylation of the p47phox subunit of NADPH oxidase, suggesting a regulatory function in ROS production.4 However, in the absence of specific inhibitors of PKCδ activity, a direct functional relevance for PKCδ in human neutrophils has not been established5 and genetic manipulation of human neutrophils is essentially impossible due to the very short life span of these cells.

Here, we have studied the role of PKCδ by using neutrophils from recently reported patients with an inherited PKCδ deficiency.6–8 These patients suffer from systemic autoimmunity and recurrent infections. Although the immunosuppressive medication to treat the autoimmune disease is a confounding factor, the patients may be intrinsically susceptible to recurrent infections.9 Our findings illustrate that PKCδ-deficient neutrophils have a markedly decreased capacity to kill bacterial and fungal pathogens. This is particularly remarkable because their ability to phagocytose, produce ROS through NADPH oxidase, as well as their capacity to release granule-derived components is virtually unimpaired. Our work provides direct evidence for the involvement of PKCδ in neutrophil microbial killing through a previously unreported nonoxidative cytotoxic mechanism.

To evaluate the function of PKCδ in human neutrophils, we used purified neutrophils from 3 patients with 2 distinct mutations in the PRKCD gene (see Table E1 in this article’s Online Repository at www.jacionline.org). Patient A, suffering from recurrent infections and autoimmunity, has a splice-site mutation in the PRKCD gene (c.1352+1G>A), leading to the lack of expression of PKCδ.11 Similarly, a complete absence of PKCδ in neutrophils from this patient was observed (Fig 1, A). In addition, we used neutrophils from 2 other patients, both carrying a homozygous missense mutation in the PRKCD gene (siblings; c.1528G>A; p.G510S), with systemic lupus erythematosus whose B cells showed minimal expression of PKCδ.7 The neutrophils of these patients expressed residual levels (~20%–30% of normal controls) of PKCδ protein (Fig 1, A). Levels of PKCα and PKCβII/I were comparable to those in controls (see Fig E1, A and B, in this article’s Online Repository at www.jacionline.org).

In neutrophils, different PKC isoforms become activated on ligation of, for instance, G-protein–coupled receptors or directly by the generic PKC agonist phorbol-12-myristate 13-acetate.4 The ability of neutrophils from PKCδ-deficient patients to activate NADPH oxidase was tested by measuring extracellular H2O2 production. PKCδ-deficient neutrophils displayed only a minor reduction in the phorbol-12-myristate 13-acetate–induced production of extracellular H2O2 (Fig 1, B) and responded completely normally to more physiologically soluble (ie, platelet-activating factor/formyl-Met-Leu-Phe) and particulate stimuli, including (opsonized) zymosan and particulate stimuli, including (opsonized) zymosan and relevant gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria and yeast (Candida albicans) (Fig 1, B and C). For comparison, neutrophils isolated

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FIG E1. Percentage of self-antigens for which there were positive IgG (A) and IgA (B) autoantibody levels. Five hundred eighty autoantibodies were demonstrated in patients with WAS and those with XLT.
FIG E2. IgG (A) and IgA (B) serum levels in multireactive and paucireactive patients with WAS/XLT.
FIG E3. A and B, Frequency of positivity for the 25 most common autoantibodies. C, RAR values for 2 IgG autoantibodies (to fibrinogen IV and mitochondrial antigen) with significantly increased levels in patients with XLT and those with WAS compared with levels in healthy control subjects (HD).