Concise Report

Abnormal apoptosis in chronic granulomatous disease and autoantibody production characteristic of lupus

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Objectives. Patients with chronic granulomatous disease and carrier mothers of patients with chronic granulomatous disease are predisposed to developing various forms of lupus. This disorder is a neutrophil defect in intracellular killing. Abnormal apoptosis has been described. We hypothesized that abnormal apoptosis occurring in neutrophils of patients made them more immunogenic.

Methods. Human patients with chronic granulomatous disease were examined for abnormalities of neutrophil apoptosis by flow cytometry. To model the effect of abnormal apoptosis, a murine model was used. Apoptotic cells from either wild type or mice with chronic granulomatous disease were injected into either wild type or chronic granulomatous disease mice and autoantibodies were determined by ELISA.

Results. Our studies found that human and murine neutrophils carrying the gp91 form of chronic granulomatous disease had impaired exposure of phosphatidyl serine on the surface. Other markers of apoptosis were largely normal. Injection of apoptotic neutrophils from gp91 knockout mice into gp91 knockout mice led to the development of characteristic autoantibodies of lupus.

Conclusions. Humans with chronic granulomatous disease may be at an increased risk of developing lupus due to abnormal apoptosis and abnormal clearance of apoptotic cells.

Key words: Lupus, Chronic granulomatous disease, Glomerulonephritis autoantibodies.

The cause of SLE is not known. People deficient in early complement components that aid in the clearance of apoptotic cells are at increased risk of SLE. Other human disorders with an increased risk of SLE include prolidase deficiency [1] and chronic granulomatous disease (CGD) [2]. The mechanisms underlying the association of prolidase deficiency and CGD with lupus are not known. Multiple murine models in which clearance of apoptotic cells is compromised develop features of lupus: Tyro 3, Axl, Mer, SAP, DNase I, immunoglobulin (Ig) M and C1q. This is consistent with studies of humans with SLE demonstrating increased lymphocyte and macrophage apoptosis and aberrant ingestion of apoptotic cells [3–5], suggesting that defective clearance of apoptotic cells may be a common prerequisite for the development of lupus.

The biochemical defect in CGD is known to lie in one of four molecules constituting the NADPH oxidase complex. The NADPH oxidase complex produces superoxide and releases it into the phagosome, where it kills bacteria. This pathway is used primarily by cells of the myeloid lineage. In a recent registry report, 2.7% of the patients with CGD developed discoid lupus (DLE) and 0.5% developed SLE [2]. While prevalence values for DLE and SLE have not been established in paediatric populations, these frequencies are substantially higher than in the general population [6]. The mothers of boys with the X-linked form were at a frequency of 8.6% and SLE at a frequency of 1%, while the mothers of children with the autosomal recessive form were not at increased risk of DLE and SLE, suggesting that having a population of cells completely deficient in NADPH oxidase contributes to the risk of SLE and DLE.

Neutrophils undergo constitutive apoptosis at a high rate and have an average lifespan of only 24 h. Approximately 25 billion polymorphonuclear neutrophils undergo apoptosis every day in an adult. Polymorphonuclear neutrophils, therefore, represent an enormous apoptotic cell burden for humans. Studies examining neutrophil apoptosis in CGD have generally demonstrated a delay in morphological manifestations of apoptosis [7–10]. One study found diminished phosphatidyl serine (PS) exposure and DNA fragmentation in CGD patients compared with controls [11].

We examined apoptosis in patients with chronic granulomatous disease in an IRB-approved study (Fig. 1). We examined PS exposure by annexin V staining (PS-positive, PI-negative) 3, 20, 24 and 32 h after drawing blood and subdiploid DNA staining with propidium iodide (PI) at the same time points. Apoptosis occurs as a result of constitutive apoptosis and integrin binding to the plastic surface [12, 13]. Six CGD patients completely deficient in gp91 and six age-matched controls were examined. The graph in Fig. 1 shows the results for annexin V staining and PI staining at 20 h. There was little change after 20 h with respect to subdiploid PI staining. The CGD samples continued to show an increase in the fraction of PS-positive cells from 20 to 32 h (from a mean of 41% PS-positive to 50% PS-positive at 32 h) while the control cells exhibited no further increase in PS-positive cells after 20 h. There was no difference in the intensity of PS staining. Terminal deoxynucleotidyl transferase biotin dUTP nick end-labelling (TUNEL) staining and trypan blue staining showed minimally decreased DNA nicking in CGD cells and no difference in trypan blue...
To determine whether the appearance of surface autoantigens was diminished along with PS exposure, we examined surface Ro by flow cytometry. Ro appears on the surface only after induction of apoptosis. We determined the percentage of Ro-positive cells within the PS-positive population. There was negligible Ro surface expression on PS-negative cells. Ro-positive cells within the PS-positive population were slightly higher in the CGD population at all time points but the difference was statistically significant only at the 3-h time point ($P = 0.03$).

These findings suggested that neutrophil apoptosis in CGD patients is aberrant and is characterized by diminished or delayed PS exposure. Recognition of PS is critical for anti-inflammatory uptake of apoptotic cells [14]. We hypothesized that neutrophils from patients with CGD might not be ingested appropriately and might immunize the person to self-antigens such as Ro. This could be relevant for the development of autoimmunity in patients with CGD. It is now believed that lupus arises in some genetically susceptible individuals years after a stimulus initiates autoantibody production [15]. We used a murine model of X-linked CGD to determine whether aberrant apoptosis could drive autoantibody production typical of lupus. This murine model has not been described as spontaneously developing lupus-like features.
We injected 10 million apoptotic thioglycollate-elicited neutrophils from either homozygous gp91 knockout (KO) mice or littermate C57BL6 mice into either female homozygous gp91 knockout mice or C57BL6 female mice for each of 4 days. The gp91KO was on the C57BL6 background. Mice were killed 6 weeks later. These thioglycollate-elicited apoptotic neutrophils exhibited the same annexin V, TUNEL and PI staining characteristics as the human CGD and control neutrophils (data not shown). Autoantibodies were assessed by enzyme-linked immunosassay (ELISA; Alpha Diagnostics San Antonio, TX, USA) and IgG deposition in the kidney was scored according to a four-point system. The gp91KO (CGD) neutrophils injected into gp91KO recipients resulted in the highest titres of autoantibodies while C57BL6 neutrophils injected into gp91KO recipients gave the next highest titres (Fig. 2). These data suggest that the apoptotic neutrophils can act as an immunogen. However, a more significant effect appears to be the presence of CGD in the recipient.

Note that, for each antibody, the two B6 recipient groups were comparable, suggesting that an intact recipient can overcome the apoptotic load of aberrant CGD cells. For each antibody, CGD recipients receiving CGD cells into had higher antibody levels than CGD recipients receiving B6 cells. Thus, there is a contribution from the immunizing cells, but in this system it was only revealed when the recipient mouse had CGD. This suggests that the NADPH oxidase system may play a role in the uptake or clearance of apoptotic material. Indeed, phagosome biochemistry is important for the degradation of DNA and failure to produce superoxide might impair the ability of the cell ingesting the apoptotic cell to digest the DNA completely [16].

Unexpectedly, glomerular IgG deposition was highest in the C57BL6 mice injected with C57BL6 cells and the gp91KO mice injected with C57BL6 cells. While there is no ready explanation for this finding, it is worth noting that human CGD patients and carriers that develop lupus seldom have renal disease. These data suggest that abnormal neutrophil apoptosis may contribute to the development of lupus but abnormal clearance of normal apoptotic neutrophils also leads to autoantibody production in this murine model.

The authors have declared no conflicts of interest.

References


