Clin Rheumatol (2003) 22: 265–267
DOI 10.1007/s10067-003-0718-1

ORIGINAL ARTICLE

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The role of apoptosis in childhood Henoch–Schonlein purpura

Received: 24 December 2002 / Accepted: 17 March 2003
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Abstract The pathogenesis of vasculitis is complex and is yet to be fully elucidated, although it is known that inflammatory cells play a major role. Dysregulation of apoptosis and defective clearance of inflammatory cells could lead to the persistence of inflammation and excessive tissue injury. In this study we aimed to investigate Fas (CD95) and apoptosis on peripheral blood (PB) neutrophil and lymphocytes in Henoch–Schonlein purpura, both in the acute phase and after resolution to determine the role of apoptosis in this self-limited vasculitis. Leukocytoclastic vasculitis presenting with Henoch–Schonlein purpura (HSP) was diagnosed according to ACR 1990 criteria and confirmed by skin biopsy. Thirty-seven patients (22 boys, 15 girls) aged 2.5–17 years (9 ± 3.3) were enrolled in the study. Expression of CD95 and apoptosis were investigated by the annexin/PI method on peripheral blood neutrophils and lymphocytes in both the acute and the resolution phases of the disease. The mean neutrophil and lymphocyte CD95 expression was 65.4 ± 37.6% and 33.3 ± 7.3%, respectively, in the acute stage and 62.8 ± 44.2% and 41 ± 20%, respectively, in the resolution ($P > 0.05$). The percentage of apoptotic peripheral blood neutrophils and lymphocytes as determined by annexin positivity was 13.3 ± 11.31% and 8.6 ± 9.5%, respectively, during the acute phase and 4.6 ± 3.4% and 3.1 ± 3.1%, respectively, in the resolution ($P = 0.002$, $P = 0.008$). These results suggest that increased apoptotic process in the immune effector cells in the acute phase of the disease may play an important role in the early control of inflammatory response and repair in leukocytoclastic vasculitis, thereby contributing to the self-limited nature of the disease.

Keywords Apoptosis · Henoch–Schönlein purpura · Pathogenesis

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Introduction

Henoch–Schönlein Purpura (HSP) is one of the most common vasculitides of childhood and mainly affects the vessels of the skin, gastrointestinal tract and kidneys. Clinically, it is characterised by non thrombocytopenic purpura, arthritis/arthralgia, abdominal pain, and gastrointestinal haemorrhage and glomerulonephritis [1]. The specific pathogenesis is still not known. In recent years cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), adhesion molecules and vascular endothelial growth factor (VEGF), have been implicated in active disease [2–4]. Apoptosis – programmed cell death – is one of the most important factors for controlling inflammation. In vasculitides, and in several other inflammatory diseases, persistent accumulation and activation of inflammatory cells is associated with tissue injury, architectural disruption and excessive fibroproliferative responses that lead to organ dysfunction and failure [5]. Failure of apoptosis and subsequent clearance processes of inflammatory cells may represent hitherto unrecognised pathogenetic mechanisms in inflammatory diseases [5].

Cross-linking of Fas and Fas ligand induces apoptosis of Fas-sensitive target cells. This pathway has been demonstrated to play an important role in the downregulation of immune response through activation-induced cell death in lymphocytes. However, its role in the resolution of non-specific inflammatory reactions has not been well documented [6, 7].

We have attempted to analyse apoptosis in childhood HSP to define its role in this self-limited vasculitis.

Patients and methods

Thirty-seven patients (22 boys, 15 girls) aged 2.5–17 years (9 ± 3.3) with a clinical diagnosis of HSP were enrolled in the study at Hacettepe University, Ihsan Doğramacı Children's Hospital, Paediatric Nephrology and Rheumatology Unit. The study was approved by ethical committee. The diagnosis of HSP was made according to ACR 1990 criteria [1] and was confirmed as leukocytoclastic vasculitis in the skin biopsy of all patients. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood cell (WBC) count were analysed both during the acute phase of the disease and after the resolution. Serum immunoglobulins and complement (C) 3 and 4 levels, which were studied by nephelometry, were also measured in the acute phase. Urinalysis was performed in all, and in those with renal involvement, renal function tests, measurement of quantitative urine protein, and creatinine clearance were determined. Five patients with renal involvement underwent renal biopsy because of either ongoing proteinuria on follow-up or rapidly progressive glomerulonephritis in the acute stage. Annexin staining was performed (described below) to study apoptosis. Fas expression was also investigated on peripheral blood neutrophils, and lymphocytes by flow cytometry.

Flow cytometry

Samples were analysed by flow cytometry for annexin V apoptosis assay and Fas cell surface expression. The annexin V binding apoptosis kit and phycoerythrin (PE)-labelled anti-CD95 monoclonal antibody and IgG_1 isotype control were purchased from Immunotech (Coulter). The analyses were performed using a Coulter Epics Elite flow cytometer.

Studies were performed at room temperature. Freshly obtained whole blood samples anticoagulated by EDTA were treated with ammonium chloride for 10 min, for red blood cell lysis. An additional treatment for a further 5 min was employed when necessary. Samples were then washed twice and subjected to monoclonal antibody incubation for 15 min. Fluorescence analysis was performed immediately after staining.

Apoptosis assay was performed according to manufacturer recommendations. Briefly, cells were washed after RBC lysis and suspended in binding buffer and incubated with 10 μl of annexin V for 10 min and 10 μl of propidium iodide (PI) for 5 min. Following two washes in PBS cells were suspended in binding buffer and analysed immediately by Coulter Epics Elite flow cytometer using standartd protocols and following instrument, alignment, optimisation and compensation procedures.

Analysis of data

All analyses were performed on peripheral blood neutrophils and lymphocytes that were gated according to forward scatter (FS) versus side scatter (SCC) properties.

The percentage of apoptotic cells, as determined by annexin positivity, was quantified. PI-positive cells were excluded regardless of their annexin staining pattern, as PI positivity indicates loss of membrane integrity and includes necrotic as well as late apoptotic cells.

Statistical analysis

Data were analysed using SPSS and results are given as median (minimum–maximum). Wilcoxon's test was used for comparing acute- and resolution-phase samples and the Mann–Whitney *U* test was used for comparing two independent samples. *P* values < 0.05 were considered significant.

Results

There were 22 boys and 15 girls with a mean age of 9 and an age range of 2.5–17 years. All patients presented characteristic palpable purpuric lesions of HSP. Twenty-five (67.6%) and nine (24.3%) had arthritis/arthralgia and gastrointestinal involvement, respectively. Twelve (32.4%) had renal involvement (haematuria and/or proteinuria). Renal biopsy was performed in five patients for proteinuria in the nephrotic range, and a diagnosis of mesangioproliferative glomerulonephritis with mesangial IgA deposition was made. One of them exhibited rapidly progressive glomerulonephritis. ESR and CRP values were high in 78% and 67% of patients, respectively, during the acute phase and returned to normal during the resolution phase (Table 1). The C3 and C4 levels were all in the normal range. Serum IgA level was increased in 64% of the patients in the acute stage. Percentage of expression of CD95 on peripheral neutrophils and lymphocytes was 78.97% and 32.51%, respectively, in the acute phase and 85.0% and 29.95%, respectively, in the resolution phase. There was no significant difference in Fas expression on neutrophils and lymphocytes between active and resolution phases (Table 2). On the other hand, neutrophil and lymphocyte apoptosis was 9.2% (1.6–45.8) and 5.5% (0.4–34.8), respectively, during the acute phase and 4.5% (0.4–12.2) and 1.8% (0.1–11.4), respectively, in the resolution ($P = 0.002$, $P = 0.008$) (Fig. 1). There was no relationship between apoptosis and/or expression of CD95 and organ involvement.

Discussion

Henoch–Schönlein purpura (HSP) is the most common vasculitis of childhood, but its pathogenesis is still not

Table 1 Serum ESR, CRP and WBC levels in all HSP patients during the acute and resolution phases

	Acute Median (min–max)	Resolution Median (min–max)	*P**
ESR (mm/h)	45 (4–104)	22 (8–55)	< 0.001
CRP (mg/dl)	1.45 (0.10–13.60)	0.20 (0.01–1.37)	< 0.001
WBC ($\times 10^9$/l)	10.5 (4.5–27.8)	7.2 (4.8–15.3)	< 0.001

*Values < 0.05 are significant

Table 2 Percentage of Fas (CD95) expression on peripheral blood neutrophils and lymphocytes during the acute and resolution phases

Fas (CD95)	Acute phase Median (min–max)%	Resolution phase Median (min–max)%	*P**
Neutrophil	78.97 (0.38–99.88)	85.00 (0.22–99.00)	0.79
Lymphocyte	32.51 (18.67–50.25)	29.95 (5.40–85.00)	0.35

*Values < 0.05 are significant

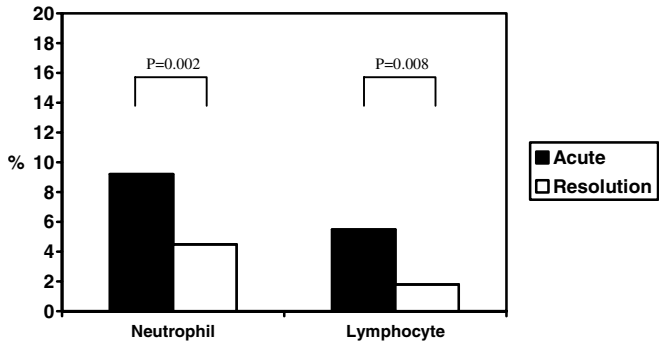

Fig. 1 Percentage of peripheral blood neutrophils and lymphocytes apoptosis during the acute and resolution phases

fully delineated. In this study we investigated the role of apoptosis of peripheral blood neutrophils and lymphocytes in the pathogenesis of HSP. Apoptosis has been investigated in a number of autoimmune and collagen vascular diseases, such as systemic lupus erythematosus. However, there are limited data in HSP [8, 9]. Increased removal of inflammatory cells by apoptosis and subsequent phagocytosis may be important for the downregulation of an inflammatory response and the prevention of tissue destruction. Fas and Fas ligand are cell surface molecules that play an important role in apoptosis. The cross-linking of Fas and Fas ligand has been shown to induce apoptosis of Fas-bearing cells [10]. In contrast, an inflammation-inducing role of Fas ligand has also been suggested in recent studies [10]. In the present study we found marked expression of Fas on peripheral blood neutrophils and lymphocytes in patients with HSP in both the acute and the resolution phases. This may suggest increased susceptibility for apoptosis to downregulate the inflammatory response in the acute phase. Moreover, persistently elevated Fas levels in the clinical recovery phase might also suggest persistent inflammation at the tissue level.

Failure of apoptosis and defective clearance of inflammatory cells may lead to persistent accumulation and activation of inflammatory cells that may be associated with tissue injury, architectural disruption and excessive fibroproliferative responses that lead to organ dysfunction and failure [5]. Apoptosis has been studied in cutaneous allergic vasculitis as well; it has been reported that there were excessive amounts of apoptosis in tissue of infiltrated cells, especially neutrophils [8]. The amount of intraglomerular Fas(+) cells was found to be high in HSP nephritis [9]. In our study increased apoptosis of peripheral blood neutrophils and lymphocytes was shown in the acute phase compared to their values in the resolution phase, perhaps suggesting a contributory role in the early control of the inflammatory response and repair in this self-limited vasculitis. The variation between patients may partly explain the interindividual variable course of the disease that is determined by the genetic factors. Further evaluation of apoptosis in non-self-limited vasculitides will clarify the importance of apoptosis in the pathogenesis of vasculitic disorders.

References

1. Cassidy J, Petty R. Vasculitis (1995) In: Cassidy J, Petty R, eds. Textbook of pediatric rheumatology, 3rd edn. Philadelphia: WB Saunders, pp. 365–422
2. Besbas N, Saatci U, Ruacan S et al. (1997) The role of cytokines in Henoch–Schönlein purpura. Scand J Rheumatol 26: 456–460
3. Söylemezoğlu O, Sultan N, Gürsel T, Buyan N, Hasanoğlu E (1996) Circulating adesion molecules ICAM-1 , E-selectin and vonWillebrand factor in Henoch–Schönlein purpura. Arch Dis Child 75: 507–511
4. Topaloglu R, Sungur A, Baskın E, Besbas N, Saatci U, Bakkaloglu A (2001) Vascular endothelial growth factor in Henoch–Schonlein purpura. J Rheumatol 28: 2269–2273
5. Haslett C (1997) Granulocyte apoptosis and inflammatory disease. Br Med Bull 53: 669–683
6. Ekert PG, Vaux DL (1997) Apoptosis and the immune system. Br Med Bull 53: 591–603
7. Alderson MR, Tough TW, Davis-Smith T et al. (1995) Fas ligand mediates activation-induced cell death in human T lymphocytes. J Exp Med 181: 71–77
8. Shi Y, Honma M, Koizumi F (1998) Cutaneous allergic vasculitis: clinicopathologic characterization and identification of apoptosis. Pathol Int 48: 705–716
9. Takemura T, Murakami K, Miyazato H, Yagi K, Yoshioka K (1995) Expression of Fas antigen and bcl-2 in human glomerulonephritis. Kidney Int 48: 1886–1892
10. Restifo NP (2000) Not so Fas: re-evaluating the mechanisms of immune privilige and tumor escape. Nature Med 6: 493–495