

Mechanism of feedback regulation of neutrophil inflammation in Henoch-Schönlein purpura

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Abstract. – **OBJECTIVE:** The aim of this study is to investigate the role of complement-neutrophil feedback regulation of inflammatory response in Henoch-Schönlein purpura (HSP) through constructing an animal model of HSP.

MATERIALS AND METHODS: Twenty-four SPF grade Japanese large-eared white rabbits were randomly divided into normal group and model group, 12 for each group. HSP model was constructed by challenging rabbits with gastric gavage of a decoction solution containing ginger, Piper longum L. and pepper, intraperitoneal injection of ovalbumin (OVA)-Freund's adjuvant and intravenous injection at marginal ear vein and subcutaneous injection in the back of rabbits with OVA normal saline solution. Changes in general conditions of rabbits including food intake, water intake and body temperature as well as alterations in blood routine, urine routine, reactive oxygen species (ROS), inflammatory cytokines and complement were compared between two groups. In the meantime, N-Acetyl-L-Cysteine (NAC) and hydrogen peroxide (H₂O₂) treatment was used to manipulate ROS level and determined the changes in aforementioned parameters.

RESULTS: After sensitization, rabbits of the model group displayed significantly elevated body temperature, apathy, reduced physical activity, significantly decreased water and food intake compared to the situations before sensitization ($p<0.05$). Significant pathological changes were observed in these rabbits through HE staining study. Furthermore, blood levels of white blood cells (WBC), mean corpuscular hemoglobin concentration (MCHC), neutrophils (NEU) and NEU% were significantly increased, whereas levels of red blood cells (RBC), hemoglobin (HGB), eosinophils (EOS) and EOS% were significantly decreased ($p<0.05$). No significant alterations were observed in levels of mean corpuscular hemoglobin (MCH) and platelet (PLT) ($p>0.05$). Urine with mucus and a strong odor was observed in model rabbits. Proteinuria occurred in 66.67% of model rabbits, hematuria in 58.33% and presence of WBC in the urine

in 25%. Also, levels of ROS, inflammatory cytokines, tumor growth factor (TGF)- β , complement and tumor necrosis factor (TNF)- α were significantly increased in model rabbits. After the treatment of ROS inhibitor, NAC, levels of these parameters were significantly decreased ($p<0.05$), but significantly increased after treatment of H₂O₂, the ROS agonist ($p<0.05$).

CONCLUSIONS: Complement-neutrophil feedback regulation of inflammatory response plays important roles in the pathogenesis of HSP, and inhibition of ROS can suppress the development and progression of HSP.

Key Words

Henoch-Schönlein purpura, Complement, Neutrophils, Inflammatory response.

Introduction

Henoch-Schönlein purpura (HSP), also known as Ashland-Hengnuo purpura, is a common form of heterogeneous, inflammatory and autoimmune vasculitis, featured by several major pathological alterations including leukocytoclastic vasculitis (LCV) in the superficial dermis, swelling of endothelial cells and infiltration of neutrophils¹. HSP is often induced via stimulation by various exogenous triggers, such as food, drugs and bacterial infection. HSP is prevalent in males. The pediatric patients aged 2-11 years can account 75% of total number of patients². However, the pathogenesis of HSP has yet been elucidated, and thereby efficient, economical, safe and targeted therapeutic strategy is not available, which makes HSP an important and urgent issue to be resolved for the treatment and prevention of dermatological diseases. Aberrant immune system and unregulated immune-inflammatory response have been considered as primary potential pathogenic mechanisms inducing HSP^{3,4}.

The complement system, known as an important component of the innate immune system, is the major effector against pathogenic invasion. In addition, a certain feedback control of inflammatory response is present between complement and neutrophils, whereby activated complement stimulates neutrophils to undergo a respiratory burst, causing the release of reactive oxygen radicals, which in turn reactivate complement. This feedback activation between complement and neutrophils enhances inflammatory responses⁵. Currently, it is unknown and has not been reported as to whether the aforementioned mechanism is involved in the regulation of the pathogenesis and progression of HSP. To this end, in the present study, an animal model of HSP was constructed in Japanese large-eared white rabbits to investigate the alterations in neutrophil-mediated inflammatory response and regulation influenced by the activation and inhibition of complement. It is expecting to provide scientific evidence for elucidating the pathogenesis of HSP.

Materials and Methods

Reagents and Instruments

Ovalbumin (OVA) and Freund's adjuvant were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. (The OVA-Freund's solution was prepared by dissolving OVA in 0.9% normal saline at a concentration of 20 mg/ml, which was mixed with Freund's adjuvant at a ratio of 1:1). Interleukin (IL)-2, IL-4, IL-6, IL-8, reactive oxygen species (ROS), transforming growth factor- β (TGF- β) and enzyme-linked immunosorbent assay (ELISA) kit for detecting complement 3 (C3), C4 and C5 were purchased from Shanghai Heng Yuan Biological Technology Co., Ltd., Shanghai, China. N-Acetyl-L-Cysteine (NAC) and H₂O₂ were purchased from Solarbio Life Sciences Co., Ltd., Beijing, China. Model 680 microplate reader was purchased from Bio-Rad Laboratories, Inc., USA. Constant temperature water bath was provided by Hebei Huanghua Instrument Plant, China. Spectrophotometer 721 model 16C14 was purchased from Shanghai Precision and Scientific Instrument Co., Ltd., Shanghai, China. Other reagents were made in China and of analytical grade.

Experimental Animals

Twenty-four healthy and SPF grade Japanese large-eared white rabbits (Laboratory Animal Cen-

ter, Sun Yat-Sen University, Guangzhou, China. Qualification certificate: SCXK(Yue)2004.001), males and females, 10 for each, aged four months, with a weight range of 2.2-3.0 kg were used to in the present study. Rabbits were numbered according to weight and randomly divided into two groups, the normal group and model group, 12 for each, male to female ratio of 1:1. Animals were bred at Laboratory Animal Center, Sun Yat-Sen University, Guangzhou, China. Animals were singly housed in each cage at a temperature of 18-25°C, relative humidity of 40%-60% and under 12h a light/dark cycle. Free access to good and water was allowed. All animal manipulations and protocols were approved by the Animal Manipulation and Care Committee.

Animal Model Construction

Sensitization via continuous antigenic stimulation

The ginger, *Piper longum* L. and pepper decoction (the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, Guangzhou, China) with a ratio of 1:1:1 was prepared in a solution with a final concentration of 15 g/100 ml. For rabbits of the normal group, a normal diet of 200 g/each rabbit/day was provided two weeks before the experiment, whereas the rabbits of the model group received gastric gavage with 1 ml of above solution once each day in addition to the normal diet. Rabbits of the normal group were gavaged with an equal volume of normal saline, and other conditions were consistent with model group. In addition, animals of the model group were challenged with an intraperitoneal injection of OVA-Freund's adjuvant solution at a dose of 1 ml/each animal/day for consecutive three weeks to achieve sensitization by continuous antigenic stimulation. Animals of the normal group were injected with equal volume of normal saline.

Allergic response to antigen challenge

After three weeks of intraperitoneal injection, animals of the model group were challenged by intravenous injection with 0.5 ml of OVA-saline solution at a concentration of 10 mg/ml via a marginal ear vein, and by subcutaneous injection with 0.2 ml of OVA-saline solution (0.3%) at five sites on the back. The success of animal model construction was confirmed by examining the irritation of the skin at injection sites after antigen challenge.

General Parameters Observed

General conditions of rabbits of two groups were examined during the experiment, including diet, water intake, body temperature, routine blood test and urine analysis. Also, 24h urine output and 24h urinary protein were measured using Coomassie Brilliant Blue method.

Determination of ROS, Inflammatory Cytokines and Complement Levels

Serum levels of ROS, IL-2, IL-4, IL-6, IL-8, TNF- β , C3, C4 and C5 were determined using ELISA. Aliquots of diluted samples were plated on 96 well microtiter plate. The standard solution was plated according to manufacturer's instruction. Samples were mixed by gentle shaking and incubated at 37°C for 30 min. After five time wash, samples were treated with enzyme labeled antibodies and incubated at 37°C for 30 min. Subsequently, samples were washed for five times, treated with substrate solution and incubate 15 min in the dark for color development. The absorbance of the sample in each well was measured at 450 nm, and the concentration of each sample was detected by a microplate reader. The levels of IL-4, IL-5, IL-8, TNF- β , C3, C4 and C5 were calculated by multiplying the concentration by the dilution factor. In the meantime, six rabbits (male:female at 1:1) from normal group and model group, respectively, were treated by intraperitoneal injection of ROS antagonist, NAC and ROS agonist, H₂O₂, in order to investigate the influence of stimulation and inhibition of ROS on serum levels of aforementioned inflammatory cytokines and complement in rabbits.

Eosin-hematoxylin (HE) Staining

Tissue samples were fixed in 95% ethanol for 20 min followed by twice wash with phosphate buffered saline (PBS), 1 min for each wash, and hematoxylin stained for 2-3 min. After washing with tap water, samples were observed under light microscope. In case nuclei were too deeply stained, 1% hydrochloric acid alcohol solution was used treat samples for several seconds to differentiate colors. Subsequently, samples were washed with tap water and stained with eosin for 1 min, washed with tap water, blow dried or air dried, and mounted with neutral resin.

Immunohistochemical Study of TNF- α Level

Rabbit tissues were routinely sectioned and dewaxed. After microwave antigen retrieval,

samples were incubated with 3% H₂O₂ for 10 min to eliminate endogenous peroxidase. Samples were then washed with PBS, 5 min each time for three times, blocked in goat serum for 30 min. After removing goat serum, samples were incubated with TNF- α antibody (1:50) for three hours at 37°C. Following PBS wash, samples were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 30min and developed using DAB. Subsequently, samples were thoroughly rinsed with water, restained with hematoxylin for 2 min, differentiated with saline alcohol, dehydrated in an alcohol gradient, cleared in xylene and mounted with neutral resin.

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation. Differences between two groups were analyzed using independent samples Student *t*-test. Comparisons between multiple groups were performed using one-way ANOVA. Non-normally distributed data or data of variance were analyzed using rank-sum test. All analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically different.

Results

Alterations in External Features of Rabbit Skin

After subcutaneous injection, skin rashes with a diameter of ~1 cm were observed at injection sites in rabbits. After 4-8 h, the skin rashes in rabbits of the normal group disappeared, whereas those in model group developed a trend toward an increasing size, and were accompanied by edema of surrounding tissue and subcutaneous bleeding. Twenty-four hours after injection, irregularly shaped cutaneous purpura with various sizes were observed in the back of rabbits of model group. Scab or necrosis occurred in some purpura during extended duration. However, no significant changes were observed in the skin of rabbits in normal group.

Changes in General Conditions of Rabbits

As presented in Table I, no significant changes were observed in the body temperature, food intake and water intake in normal rabbits before and after sensitization ($p > 0.05$). Conversely, after

Table I. Changes in body temperature, food and water intake in rabbits of model group and normal group.

Group	Body temperature (°C)		Food intake (g)		Water intake (ml)	
	Before sensitization	After sensitization	Before sensitization	After sensitization	Before sensitization	After sensitization
Normal group	38.41±0.19	38.58±0.18	200.45±10.63	197.56±12.78	308.25±22.93	319.58±21.45
Model group	38.13±0.22	39.05±0.24* [#]	201.82±12.05	163.92±9.84* [#]	306.81±20.76	286.52±14.93* [#]
<i>t</i>	1.491	4.591	1.057	5.190	0.985	12.582
<i>p</i>	0.293	0.028	0.314	0.001	0.496	<0.001

*Vs. before sensitization, $p<0.05$, [#]Vs. normal group, $p<0.05$

sensitization, rabbits of model group displayed apathy, reduced physical activity, significantly decreased water and food intake compared to the situations before sensitization ($p<0.05$). Compared with normal group, rabbits of model group presented significantly elevated body temperature after sensitization ($p<0.05$), but significantly decreased food and water intake ($p<0.05$).

Changes in the Results of Blood Routine and Urine Routine

As shown in Table IIA and IIB, no significant differences were observed in the results of blood routines before sensitization between model group and normal group ($p>0.05$), and no significant alteration was observed in the blood routine of normal rabbits after sensitization ($p>0.05$). However, in sensitized model rabbits, levels of white blood cells (WBC), *mean corpuscular hemoglobin concentration* (MCHC), neutrophils (NEU) and NEU% were significantly increased. Whereas levels of red blood cells (RBC), hemoglobin (HGB), eosinophils (EOS) and EOS% were significantly decreased ($p<0.05$). No significant alterations were obser-

ved in levels of *mean corpuscular hemoglobin* (MCH) and *platelet* (PLT) ($p>0.05$). In addition, urine routines of rabbits of both groups were normal before sensitization. And no significant changes occurred in urine routine of normal rabbits after sensitization. However, urine with mucus and a strong odor was observed in model rabbits. Proteinuria occurred in 66.67% of model rabbits ($n=8$), hematuria in 58.33% ($n=7$) and WBC in the urine in 25% ($n=3$). No significant changes were observed in 24h urine output, urine glucose, bilirubin, ketones, urobilinogen, urine specific gravity (SG) and pH value. However, 24h urine protein was increased from 103.25 mg/24h to 402.45 mg/24h with statistical significance ($p<0.05$).

Alterations in Levels of ROS, Inflammatory Cytokines and Complement in Rabbits

As demonstrated in Table IIIA and IIIB, before sensitization, no significant differences were observed in the levels of ROS, IL-2, IL-4, IL-6, IL-8, TGF- β , C3, C4 and C5 between rabbits of normal group and model group ($p>0.05$). After sensitization, no significant changes occurred in

Table IIA. Changes in blood routines of rabbits in model group and normal group.

Group	WBC ($10^{12}/L$)		RBC ($10^{12}/L$)		HGB (g/L)		MCH (fL)		MCHC (g/L)	
	BS	AS	BS	AS	BS	AS	BS	AS	BS	AS
Normal group	6.81±1.23	7.20±1.85	5.55±0.68	5.93±0.89	105.24±7.58	104.29±7.04	19.45±0.84	20.45±0.92	275.32±4.98	278.49±4.09
Model group	6.90±1.18	22.59±5.68* [#]	5.61±0.77	4.09±0.76* [#]	104.98±8.04	92.58±6.45* [#]	19.08±0.92	20.88±0.86	274.76±5.32	306.82±5.98* [#]
<i>t</i>	1.693	17.192	0.878	4.058	0.875	10.581	0.572	1.752	0.626	8.482
<i>p</i>	0.235	<0.001	0.580	0.036	0.582	<0.001	0.803	0.227	0.739	<0.001

BS = Before sensitization; AS = After Sensitization; *Vs. before sensitization, $p<0.05$, [#]Vs. normal group, $p<0.05$

Table IIB. Changes in blood routines of rabbits in model and normal groups.

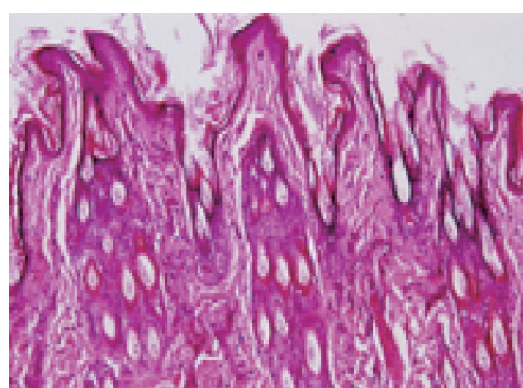
Group	PLT (10 ⁹ /L)		NEU (10 ⁹ /L)		NEU (%)		EOS (10 ⁹ /L)		EOS (%)	
	BS	AS	BS	AS	BS	AS	BS	AS	BS	AS
Normal group	332.45± 11.95	334.46± 10.85	0.18± 0.04	0.20± 0.08	2.45± 0.92	2.52± 0.89	1.88± 0.79	1.86± 0.76	27.39± 5.23	26.93± 4.94
Model group	330.83± 12.34	335.38± 11.24	0.19± 0.06	0.61± 0.13*#	2.53± 0.89	3.15± 0.95*#	1.86± 0.68	1.45± 0.51*#	27.45± 5.57	20.48± 5.29*#
<i>p</i>	0.678	1.294	0.792	5.192	1.231	4.928	0.728	4.004	1.265	6.792
<i>p</i>	0.782	0.301	0.856	0.002	0.294	0.011	0.803	0.032	0.203	<0.001

BS = Before sensitization; AS = After Sensitization; *Vs. before sensitization, $p<0.05$, #Vs. normal group, $p<0.05$

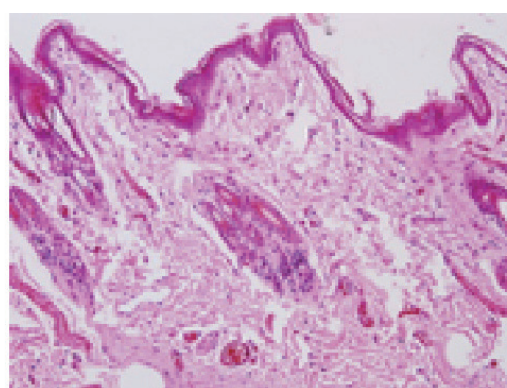
the levels of above parameters in normal rabbits. However, levels of ROS, inflammatory cytokines, TGF- β and complement were significantly increased in model rabbits and were significantly higher than those in normal rabbits ($p<0.05$). Furthermore, after the treatment of ROS inhibitor, NAC, levels of ROS, inflammatory cytokines, TGF- β , C3, C4 and C5 were significantly decreased ($p<0.05$), but significantly increased after treatment of H₂O₂, the ROS agonist ($p<0.05$).

HE Staining

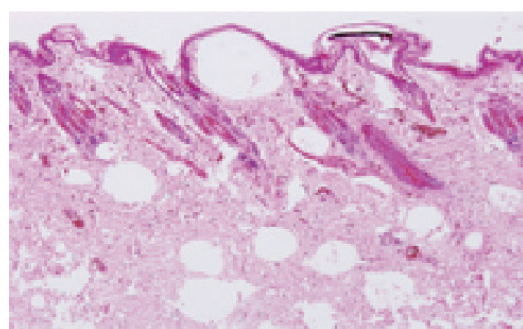
Various pathological alterations were observed in the skins of model rabbits, including significant ballooning degeneration, intercellular edema, widening of intercellular gap, stretched intercellular bridge and cellular swelling (Figure 1). Also, these pathological changes were aggravated when ROS production was promoted. There were significant improvements when ROS was inhibited in model rabbits.



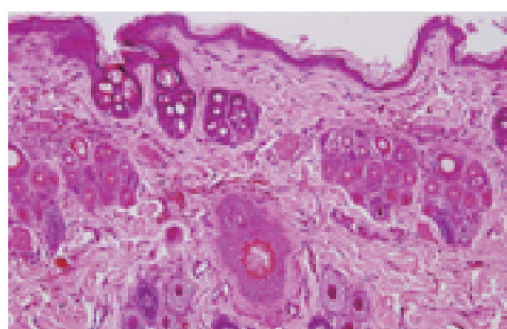
Normal group



Model group



ROS promoted model group



ROS inhibited model group

Figure 1. HE staining of skin tissue of rabbits (200 $\times$).

***TNF- α* Expression**

Compared to normal controls, TGF- α expression levels were significantly higher in the kidneys and skin tissues of model rabbits, and TGF- α levels were significantly increased when ROS was promoted; conversely, ROS inhibition significantly reduced TGF- α expression (Figure 2A and 2B).

Discussion

To our knowledge, the present study is the first study of detailed pathogenesis of HSP using large-eared rabbit as an animal model. The ani-

mal model of HSP was constructed by sensitizing rabbits with continuous antigen stimulation using OVA-Freund's complete adjuvant, which provided a practical experimental model and research platform for the study of the pathogenesis of HSP. In addition, further study⁶ was performed to investigate the regulation and alteration of neutrophil-mediated inflammatory response to the activation and inhibition of ROS, which has not been reported to date. The results of the present study showed that in HSP model rabbits, levels of inflammatory cytokines including IL-2, IL-4, IL-6, IL-8 and TGF- α were significantly increased,

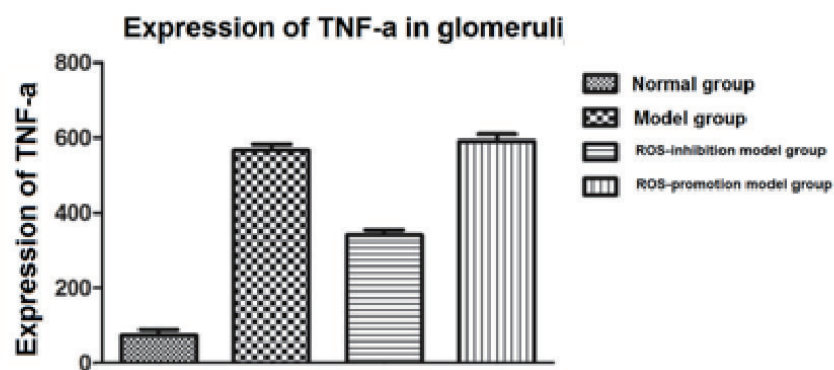
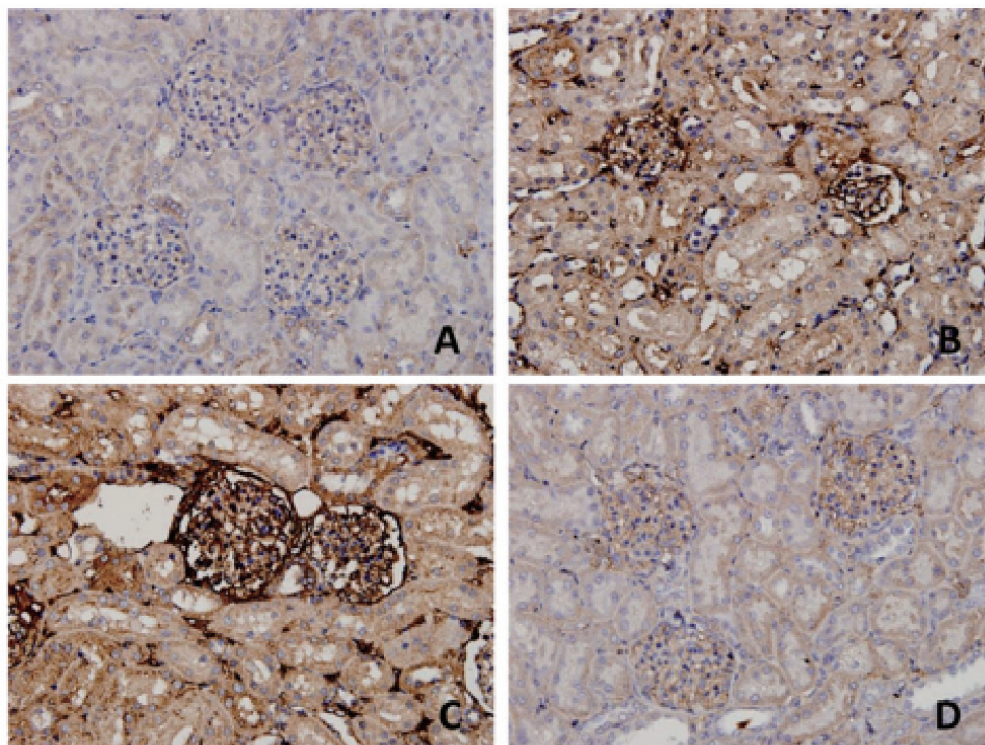


Figure 2A. Determination of TNF- α expression level in rabbit kidney using immunohistochemical study (400 $\times$). **A**, Normal group; **B**, Model group; **C**, ROS promoted-model group; **D**, ROS inhibited-model group.

and were decreased when ROS was inhibited. However, more significant inflammatory response was observed when ROS was stimulated, fully demonstrating that antagonism against ROS level could attenuate the neutrophil-mediated inflammatory response. Moreover, when inflammatory reaction became intensified, significant changes in the results of blood routine, such as levels of WBC, MCHC, NEU and NEU%, were observed in model rabbits, which served an adaptation to the inflammation. These changes resulted in poor mental state, loss of appetite and reduced physical activity and other symptoms in model rabbits.

In addition, the results of HE staining showed significant pathological alterations in skin tissue of model rabbits, which was effectively improved upon ROS inhibition, indicating that inhibition of ROS not only can improve inflammatory parameters in rabbits with HSP, but also can alleviate symptoms of the disease and pathological phenotype.

Currently, it has been widely believed that inflammatory disorders, especially diseases with the presence of immune complexes (such as HSP, etc.), tissue response to inflammatory injury is primarily mediated by the activation of com-

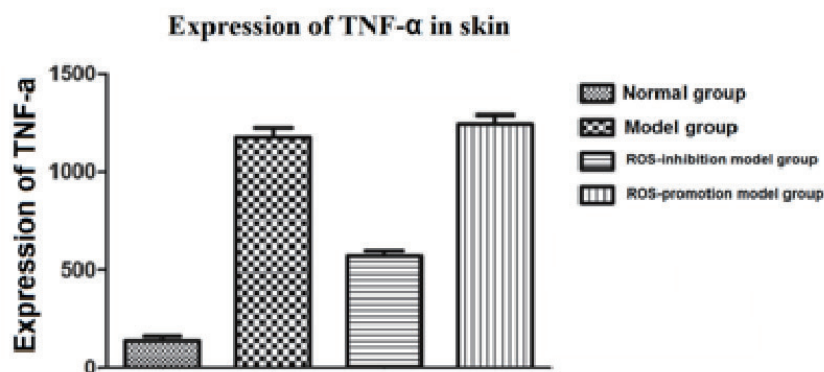
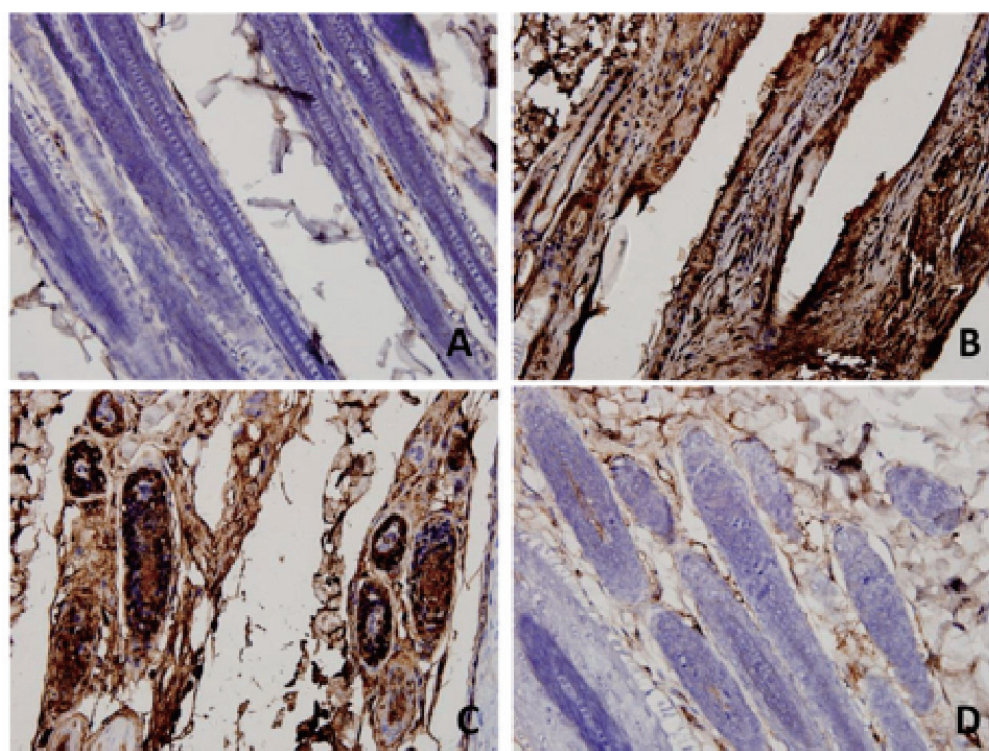


Figure 2B. Determination of TNF- α expression level in rabbit skin tissue using immunohistochemical study (400 $\times$). **A**, Normal group; **B**, Model group; **C**, ROS promoted-model group; **D**, ROS inhibited-model group.

plement system^{7,8}. For decades, the relationship between complement activation and inflammatory injury has been receiving much attention from many clinical researchers. A study⁹ has shown that activated complement can produce anaphylactic peptides, which in turn induce neutrophils chemotaxis, and induce the release of lysosomal enzymes of WBC under the action of cytochalasin B. Animal studies have also confirmed that inhibition of complement activation or oxygen free radicals scavenging can effectively alleviate the symptoms of arterial inflammation in animal skin. Furthermore, activated complement fragment can induce neutrophil chemotaxis and stimulate a cascade of reactions, resulting in the release of ROS¹⁰. The results of the present study also showed that levels of C3, C4 and C5 were significantly elevated in model rabbits, suggesting that complement system was activated during the development of HSP. More importantly, we also found that inhibition of ROS level could attenuate the activation of complement and reduce inflammatory reactions, indicating that complement-ROS feedback regulation system is involved in the genesis and progression of HSP.

Foreign researchers have also reported that neutrophil activation during inflammatory injury and the oxidative metabolites produced by activated neutrophils is an important pathway of complement activation. In addition, neutrophils and complement are mutually reinforced through a feedback regulation, whereby complement activation induces respiratory burst in neutrophils during inflammatory injury, and free radicals produced by neutrophils can also promote the activation of complement^{11,12}. This feedback control is very likely to be an important pathway to regulate inflammatory response and injury. Furthermore, during the inflammation of organs and blood vessels, the expression of TNF, IL, TGF- β , TGF- α and other inflammatory cytokines were increased. These cytokines can also be massively produced by neutrophils and induce tissue injury. Besides, these cytokines can, in turn, activate more production of neutrophils, resulting in a vicious cycle^{13,14}. Upon complement activation, inflammatory cytokines can also stimulate the production and release of neutrophils from the bone marrow, increase the expression of adhesion molecules, accelerate the invasion of neutrophils into tissue, induce respiratory burst and degranulation of neutrophils, promote the production of plasminogen activator inhibitor from endothelial cells and finally lead to the occurrence of vasculitis^{15,16}.

Conclusions

In the present study, a complement-neutrophil feedback regulation of inflammatory response is shown to play important roles in the pathogenesis of HSP through constructing an animal model of HSP. In the meantime, this study also demonstrated that the genesis and progression of HSP can be inhibited by ROS intervention through bidirectional regulation of ROS using NAC and H₂O₂. These results sufficiently demonstrated that the application of ROS inhibitor may have positive effect in improving the symptoms of HSP, controlling inflammatory reactions in patients and promoting the prognosis of the disease.

Conflicts of interest

The authors declare that no conflicts of interest exist.

References

- 1) YANG YH, YU HH, CHIANG BL. The diagnosis and classification of Henoch-Schönlein purpura: An updated review. *Autoimmunity Rev* 2014; 13: 355-358.
- 2) TAYABALI S, ANDERSEN K, YOONG W. Diagnosis and management of henoch-schönlein purpura in pregnancy: a review of the literature. *Arch of Gynecol Obstet* 2012; 286: 825-829.
- 3) KIRYLUK K, MOLDOVEANU Z, SANDERS JT, EISON TM, SUZUKI H, JULIAN BA, NOVAK J, GHARAVI AG, WYATT RJ. Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch-Schönlein purpura nephritis. *Kidney Int* 2011; 80: 79-87.
- 4) JEN HY, CHUANG YH, LIN SC, CHIANG BL, YANG YH. Increased serum interleukin-17 and peripheral Th17 cells in children with acute Henoch-Schönlein purpura. *Pediatr Allergy Immunol* 2011; 22: 862-868.
- 5) PARK SJ, SUH JS, LEE JW, KIM SH, HAN KH, SHIN JI. Advances in our understanding of the pathogenesis of Henoch-Schönlein purpura and the implications for improving its diagnosis. *Expert Rev Clin Immunol* 2013; 9: 1223-1238.
- 6) ZHANG Q. The immune abnormalities of Henoch-Schönlein purpura. *Int J Immunol* 2007; 30: 62.
- 7) TAHAN F, DURSUN I, POYRAZOGLU H, GURGOZE M, DUSUNSEL R. The role of chemokines in Henoch Schonlein Purpura. *Rheumatol Int* 2007; 27: 955-960.
- 8) McDONALD B, PITTMAN K, MENEZES GB, HIROTA SA, SLABA I, WATERHOUSE CC, BECK PL, MURUVE DA, KUBES P. Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science* 2010; 330: 362-366.
- 9) ZIPFEL PF, SKERKA C. Complement regulators and inhibitory proteins. *Nat Rev Immunol* 2009; 9: 729-740.

- 10) MANTOVANI A, CASSATELLA MA, COSTANTINI C, JAILLON S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* 2011; 11: 519-531.
- 11) CHOU RC, KIM ND, SADIK CD, SEUNG E, LAN Y, BYRNE MH, HARIBABU B, IWAKURA Y, LUSTER AD. Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. *Immunity* 2010; 33: 266-278.
- 12) PHILLIPSON M, KUBES P. The neutrophil in vascular inflammation. *Nat Med* 2011; 17: 1381-1390.
- 13) FOX S, LEITCH AE, DUFFIN R, HASLETT C, ROSSI AG. Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease. *J Innate Immun* 2010; 2: 216-227.
- 14) PAONE G, CONTI V, LEONE A, SCHMID G, PUGLISI G, GIANNUNZIO G, TERZANO C. Human Neutrophil Peptides sputum levels in symptomatic smokers and COPD patients. *Eur Rev Med Pharmacol Sci* 2011; 15: 556-562.
- 15) WANG R, LU B, GERARD C, GERARD NP. Disruption of the complement anaphylatoxin receptor C5L2 exacerbates inflammation in allergic contact dermatitis. *J Immunol* 2013; 191: 4001-4009.
- 16) ZHANG J, KOH J, LU J, THIEL S, LEONG BS, SETHI S, HE CY, HO B, DING JL. Local inflammation induces complement crosstalk which amplifies the antimicrobial response. *PLoS Pathog* 2009; 5: e1000282.