Elevation of Neutrophil CEACAM1 Associated with Multiple Inflammatory Mediators was Related to Different Disease Progression in Ischemic Stroke Patients

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Research

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Abstract

Background

Tissue damage, expecially blood-brain barrier (BBB) injury caused by inflammatory factor storm and stroke-associated infection (SAI) during cerebral stroke is an important factor affecting the prognosis of patients. We aim to analyze the level of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) together with the multiple inflammatory mediators at different stages of ischemic stroke (IS), as well as to investigate the role of CEACAM1 and the potential underlying mechanism in IS in the meanwhile.

Methods

The frequency of CEACAM1 positive neutrophils, neutrophil viability and levels of intracellular cytokines were detected by flow cytometry. The plasma CEACAM1, neutrophil gelatinase-associated lipocalin (NGAL), matrix metalloproteinases-9 (MMP-9), interleukin-10 (IL-10) and tumor necrosis factor (TNF) were measured by ELISA.

Results

Compared with the normal control, the percentage of CEACAM1 positive neutrophils in the peripheral blood in the IS patients showed significant elevation, and a significant increase was also noticed in the content of plasma CEACAM1 at the subacute stage. There was a positive correlation between CEACAM1 expression rate in the neutrophils and plasma CEACAM1 and IL-10 content in the subacute group. Compared with the acute group and healthy control group, there was an instinct elevation in the level of plasma MMP-9 in the subacute group. The plasma NGAL in the subacute and stable groups was significantly higher than that of the normal control.

Conclusion

Our data showed that there was rapid elevation of CEACAM1 in the neutrophils at the acute stage of cerebral IS. We speculated that CEACAM1 may serve as an inhibitory regulator involved in the progression of focal cerebral ischemia. Moreover, the elevation of serum NGAL at the acute and stable stages may involve in the BBB injury mediated by MMP-9.

Introduction

Stroke, with an estimated global lifetime risk of 24.9%, shows an increasing trend worldwide[1]. As the third major cause for disability-adjusted life-years[2], stroke shows a high mortality and morbidity, which is a severe threat to public health[3]. Ischemic stroke (IS), accounting for 80%-85% of the stroke

population, is mainly associated with several factors, especially the thromboembolic occlusion in the cerebral artery. Upon onset of stroke, many patients present hypoxic damages and excitotoxicity. Subsequently, a majority would show massive accumulation and infiltration of inflammatory cells during ischemic reperfusion that may trigger further injuries to the brain tissues[4, 5]. Among these inflammatory factors, blood-brain barrier (BBB) injury is crucial for the pathogenesis of tissue damages in IS. Nevertheless, in order to antagonize the injuries to the BBB and the nervous system induced by excessive inflammatory reactions, the human body would persistently release the anti-inflammatory signals to defend the excessive inflammation to maintain the homeostasis. With the increase of anti-inflammatory factors, the immune system showed gradual failure, which finally entered into systemic immunosuppression[6–8], possibly leading to stroke-associated infection (SAI)[9, 10]. Susceptibility to infection may severely hamper the prognosis, and is mainly responsible for the elevation of mortality among stroke patients[6, 11]. On this basis, cerebral IS patients may present immunological function disorders, especially the functional disorder of the innate immunocytes associated with the early-stage BBB injury and late-stage SAI. Nevertheless, little is known about the exact mechanisms in this process.

Neutrophils are the major effector cells of the innate immunity system. Upon IS onset, the neutrophils were the first type of cells adhered to the cerebral endothelium and to infiltrate to the ischemic brain in the presence of cell adhesion molecules and chemotactic factors[12]. Besides, it would lead to BBB and nervous system injuries through releasing the inflammatory mediators including proinflammatory cytokines, matrix metalloproteinases, as well as reactive oxygen species[13]. Evidence showed that after IS, the circulating leukocytes would infiltrate into the lesion sites in the presence of pro-inflammatory factors and chemotactic factors, which then further led to BBB injury through releasing proinflammatory cytokines, and reactive oxygen species and MMPs, together with formation of the vasogenic edema[14–16]. In the previous study, MMP-9 was considered to play an important role in the BBB injury after stroke, while the MMP-9 inhibitor provided protection against potential alternations in BBB permeability[17, 18]. Furthermore, elevation of serum IL-10 and MMP-9 was observed in patients with stroke, and IL-10 was found to play an important anti-inflammatory role in response to brain injury[19]. However, MMP-9 might be a potential trigger for inflammatory response, and it was correlated with a poor outcome in ischemic stroke [20–22]. Previously, neutrophils were considered the major source of MMP-9 after stroke[23]. In a recent study, CEACAM1 was reported to involve in controlling the secretion of MMP-9 by neutrophils in post-ischemic inflammation in BBB after stroke[24]. This implied that CEACAM1 could mediate tissue damages and modulate the breakdown of BBB in focal cerebral ischemia. According to the previous study, CEACAM1 played a crucial role in the immune regulation[25]. As a member of the immunoglobulin superfamily, CEACAM1 is known as an inhibitory immune co-receptor suppressing the signal transmission via two immunoreceptor tyrosine-based inhibition motifs[25, 26]. In a mice model of IS, CEACAM1 could obviously inhibit the MMP-9 mediated BBB injury, while in the CEACAM1$^{-/-}$ IS mice, there were increased stroke volumes, BBB breakdown and high post-stroke-associated mortality. Whereas, little is known about the correlation between CEACAM1 expression and MMP-9 in the peripheral blood of IS patients. Moreover, it is closely related to the proliferation and apoptosis of neutrophils and lymphocytes[27–29], together with the secretion of cytokines[30, 31]. Our previous study indicated that

there was significant elevation of $CD8^+$ T cell TIM-3 in the stroke patients[32], and CEACAM1 was also one of the ligands for TIM-3[33]. NGAL is reported to be involved in the innate immunity, cellular differentiation and proliferation, as well as iron homeostasis[34]. Besides, it may form the complex with MMP-9[35]. Thus, CEACAM1 could directly or indirectly be involved in the inherent and regulatory immunity. In this study, we aimed to analyze the level of neutrophils and the serum CEACAM1, MMP-9 and NGAL content in the stroke patients, and analyze their possible roles in the stroke.

Material And Methods

Study subjects

Sixty-seven IS patients admitted in the First Affiliated Hospital, College of Medicine, Zhejiang University were recruited in this study. The diagnosis of IS was conducted using criteria issued by the World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (WHOMONICA). IS was verified by magnetic resonance imaging (MRI) or computed tomography (CT). All the patients were classified into the following groups according to the time of post-stroke: (i) acute phase group (within post-IS 48 h); (ii) sub-acute phase group (post-IS, 48 h to 10 days), and (iii) stable phase group (post-IS, 10–30 days). Clinical data including gender, race, age, alcohol use status, smoking status, other disease status, and the National Institutes of Health Stroke Scale (NIHSS) score were recorded for the stroke subjects. Infection was defined as presence of clinical symptoms including pyuria for urinary tract infection, fever, productive cough, positive bacterial culture, as well as radiographic evidence of consolidation for pneumonia. These who met the exclusion criteria were excluded from this study: those with a history of cerebral abscess, cerebral hemorrhage, transient ischemic attack, hematopathy, autoimmune diseases, cancer and pregnancy, trauma or surgery within the last 3 months, severe infection in the recent 6 months, together with a history of HIV infection. Patients with liver and renal dysfunction were excluded from this study. In addition, we included 20 healthy individuals who had received physical examination in the First Affiliated Hospital of Zhejiang University at the same time period as normal control. Table 1 summarized the demographic and clinical characteristics of subjects. Written informed consent was obtained from each subject.

Table 1
Demographics and baseline characteristics

Variables	Control group (n = 20)	Acute phase group (n = 19)	Sub-acute phase group (n = 28)	Stable phase group (n = 20)
Age (years)	55.75 ± 11.36	58.26 ± 16.10	68.11 ± 11.59	66.67 ± 11.74
Male %	55.00%	52.63%	60.71%	55.00%
Race, Han, % (n)	100	100	100	100
NISSH score	NA	6.11 ± 4.03	3.47 ± 2.05	1.17 ± 1.17
Smoking status (% current smokers)	30.00	42.11	39.29	40.00
Hypertension, % (n)	0.00	63.16	67.86	65.00
Diabetes mellitus, % (n)	0.00	36.85	32.14	35.00
Hypercholesterolemia, % (n)	0.00	50.00	53.58	40.00

Flow cytometry

Figure 1A showed the gating strategy of different immune cell populations. Briefly, the frequencies of lymphocytes, neutrophils and monocytes in the leukocyte population were determined based on their CD45 (APC BD Bioscience)-SSC profile. T lymphocytes were $CD45^+CD3^+$ (BV421 BD Bioscience), and B lymphocytes were $CD45^+CD19^+$ (FITC Biolegend). The NK cells were $CD45^+CD3^-CD16^+$ (APC, BD Bioscience). Monocytes were $CD45^+CD14^+$ (FITC BD Bioscience). The cells were analyzed using CEACAM-1 (PE R&D). Isotype-matched immunoglobulins served as control. About 30 min later, TQ-PREP (Beckman Coulter) was used to lyse the heparinized blood. Flow cytometry (FACS CANTO II, Becton Dicknson) was used for the analysis of blood samples in this study.

Cell viability

The heparinized blood was lysed using TQ-PREP before the neutrophils were labelled with Annexin V-FITC (BD Bioscience) and CEACAM1 (PE R&D). Flow cytometry was utilized to detect the CEACAM1 positive cells and the cells negative for apoptosis (FACS CANTO II, Becton Dicknson).

Measurement of plasma CEACAM-1, NGAL and MMP-9

The concentrations of plasma or poor-plate-plasma (targeted MMP-9) were determined using a commercial ELISA Kits. CEACAM-1 (DY2244) and MMP-9 (DMP900) were purchased from R&D, while the NGAL (443407) was purchased from Biolegend. The absorbance was determined at 450 nm using an

ELISA reader (iMark Microplate Reader, Bio-Rad). The limitation of detection for CEACAM-1, MMP-9 and NGAL was 93.8 pg/ml, 0.156 ng/ml, and 16.4 pg/ml, respectively.

Intracellular MMP-9 and cytokine assays

The neutrophils were labelled with CEACAM1-PE (R&D) at room temperature for 30 min, and then were resuspended with 1 ml PBS. LPS (100 μg/ml, Sigma-Aldrich) and Brefeldin A (BFA, Biolegend) was used to inhibit cytokine trafficking. About 2 h after incubation, the cells were fixed and permeabilized (Biolegend), followed by staining with MMP-9-FITC (R&D), TNF-α-APC and Il-10-BV421 (Biolegend) at room temperature for 30 min. Finally, flow cytometry was conducted to analyze the intracellular MMP-9 and cytokines.

Plasma cytokine assays

The concentrations of plasma TNF and IL-10 were determined using a Human Enhanced Sensitivity Flex set (BD Bioscience). The lower limits of the test for the detection of the cytokines were 67.3 fg/mL for TNF and 13.7 fg/mL for IL-10.

Statistical Analysis

We used the SPSS 22.0 software for the data analysis. The data were presented as the mean ± standard deviation. The normal distribution of data was determined based on the descriptive statistics. One-way analysis of variance (ANOVA) was utilized for the comparison of data that were normally distributed. Differences between groups were determined using the post hoc test. Kruskal-Wallis was utilized for the analysis of data that were not normally distributed. The difference of independent data between two groups was analyzed by the Mann–Whitney nonparametric test. Pearson correlation analysis was performed to assess associations among plasma TNF, IL-10, CEACAM1 content and CEACAM1 positive neutrophils in the IS group. Statistical difference was considered in the presence of $P < 0.05$.

Results

Frequency of CEACAM1-positive cells on the surface of neutrophils in the peripheral blood

Flow cytometry validated the expression of CEACAM1 in the white blood cells of peripheral blood in stroke patients. CEACAM1 was mainly expressed on the surface of neutrophils. In contrast, $CD3^+$ T cells, NK cells, monocytes and B cells expressed low levels of CEACAM1 (Fig. 1A and 1B). As shown in Fig. 1C and 1D, the percentage of CEACAM1-positive cells on the surface of neutrophils in the peripheral blood in IS groups showed significant increase compared with that of the control group ($P < 0.01$). There were no statistical differences in the percentage of CEACAM1-positive cells on the surface of neutrophils among the acute group, subacute group and stable group ($P > 0.05$).

Apoptotic phenotype of CEACAM1 positive and negative neutrophils

To confirm the correlation between CEACAM1 expression and the apoptosis of neutrophils, flow cytometry was utilized to determine the positive rate of Annexin-V in the CEACAM1 positive and negative neutrophils. As shown in Fig. 2A and 2B, in patients at subacute stage, Annexin-V was expressed in both CEACAM1 positive and negative neutrophils. However, there were no statistical differences in the ratio of CEACAM1 positive and negative neutrophils ($P > 0.05$).

Determination of plasma soluble CEACAM1

In this part, we determined plasma CEACAM1 levels among IS groups and the control group. The level of plasma CEACAM1 in the subacute group showed significant increase compared with that in the normal control group ($P = 0.016$, Fig. 3A). No statistical differences were noticed between subacute group and the other groups ($P > 0.05$). Moreover, the expression of CEACAM1 on the surface of neutrophils in the subacute group was closely correlated with the plasma CEACAM1 content ($P = 0.001$, $r = 0.614$, Fig. 3B).

Determination of poor-plate-plasma MMP-9 and NGAL

We determined the plasma MMP-9 concentration in different groups. Significant elevation was noticed in the plasma MMP-9 concentration in the subacute group when comparing with that of the control group ($P = 0.005$) and acute group ($P = 0.020$, Fig. 3C). There were no statistical differences among the other groups ($P > 0.05$).

We also determined the level of plasma NAGL in different groups. Compared with the normal control individuals, significant elevation was noticed in the plasma NAGL in the subacute group ($P = 0.001$) and stable group ($P < 0.001$, Fig. 3D). There were no statistical differences in the plasma NAGL between the other groups ($P > 0.05$).

TNF-α, MMP-9 and IL-10 production by CEACAM1 positive and negative neutrophils

To confirm the correlation between expression of CEACAM1 and the production of MMP-9, TNF-α and IL-10 of neutrophils, the neutrophils were subjected to LPS stimulation among the patients from the subacute group. Flow cytometry was utilized to determine the mean fluorescence intensity (MFI) of TNF-α, MMP-9 and IL-10 in the CEACAM1 positive and negative neutrophils. As shown in Fig. 4, TNF-α, MMP-9 and IL-10 were expressed in both CEACAM1 positive and negative neutrophils, but there were no statistical differences in the MFI of the two subtypes of neutrophils ($P > 0.05$).

Determination of plasma soluble TNF and IL-10

We also determined the level of plasma TNF and IL-10 in different groups. Significant elevation was noticed in the plasma IL-10 concentration in the subacute group when comparing with that of the control group ($P = 0.012$) and acute group ($P = 0.012$, Fig. 5A). There were no statistical differences among the other groups ($P > 0.05$). Moreover, the expression of CEACAM1 on the surface of neutrophils in the acute and subacute group was closely correlated with the plasma IL-10 content ($P = 0.009$, $r = 0.582$ and $P = 0.009$, $r = 0.482$ respectively,Figure 5C and 5D). There were no statistical differences in the concentration

of TNF among the different groups ($P > 0.05$, Fig. 5B) and TNF contents in all the groups were not correlated with levels of CEACAM1 expression in neutrophils.

Discussion

Cerebral edema after BBB breakdown is considered an important cause for the severe morbidity and mortality after IS, serving as an important cause for the early stroke-related death[36]. Besides, SAI is the major cause for the late-stage mortality in IS[37]. Increasing evidence suggested that functional disorder of the immunocytes was an important cause for the early stage BBB injury and subsequent SAI among patients with stroke. However, the roles of neutrophils in the pathogenesis of stroke have not been well defined. In this study, we measured the expression of serum CEACAM1, MMP-9 and NGAL content, as well as the expression of CEACAM1 in the neutrophils. The serum CEACAM1, MMP-9 and NGAL in the IS patients showed significant increase, and there was also significant elevation in the counts of CEACAM1 positive neutrophils. These features of CEACAM1, MMP-9 and NGAL may represent an unknown mechanism associated with the pathogenesis of IS.

According to previous works, MMP-9 was implicated in the BBB breakdown and formation of vasogenic edema after stroke[38, 39]. Consistently, our results showed that the serum MMP-9 level in patients at subacute phases was significantly higher than that of the acute stage and the normal control. Interestingly, there were no statistical differences in the plasma MMP-9 between the normal control and the acute group. This implied that there was persistent elevation of MMP-9 within several days after onset of IS, which may be closely related to the subsequent brain edema and nervous injuries.

In an IS mice model, CEACAM1 was involved in controlling the secretion of MMP-9 by neutrophils in postischemic inflammation in the BBB after stroke[24]. This demonstrated that CEACAM1 may inhibit the neutrophil-mediated tissue damages and breakdown of BBB in focal cerebral ischemia. In this study, we determined the expression of CEACAM1 in leukocytes of IS patients. Our data confirmed that neutrophils were major source of CEACAM1 expression in the peripheral blood. CEACAM1 expression on the surface of peripheral neutrophils of IS patients at each stage was significantly higher than that of the normal control. Meanwhile, the plasma CEACAM1 and MMP-9 in IS patients also showed significant changes. Ludewig Peter and his colleges described a significant increase of the expression of MMP-9 in $CEACAM1^{-/-}$ IS mice model compared with that of the wild type mice and the results were validated with stimulation of neutrophils under *in vitro* conditions showing increased MMP-9 secretion in CEACAM1-deficient neutrophils 120 min afterwards[24]. These results suggested that elevation of CEACAM1 in stroke patients was possibly closely related to BBB injury mediated by MMP-9. Moreover, it has been reported that CEACAM1 inhibited the inflammatory response induced by formation of toll-like receptor 4-CEACAM1 complex on neutrophils responding to pathogen challenge, which resulted in reduction of proinflammatory cytokine (e.g. IL-1β) [30]. These results indicated that the expression of CEACAM1 in the neutrophils regulated the expression of MMP-9 and proinflammatory factors. Nevertheless, in this study, upon LPS stimulation, no statistical differences were noticed in the MMP-9 expression in CEACAM1 positive and negative neutrophils in IS patients. Our data showed that neutrophil CEACAM1 expression

showed no influence or regulatory effect on viability or function of neutrophils. On this basis, we speculated that CEACAM1 may play a role in pathophysiology of IS through other pathways. For instance, Pan et al reported that CEACAM1 acted as a co-inhibitory receptor for G-CSFR regulation granulopoiesis[28]. CEACAM1 regulated the vascular homeostasis and integrity, which resulted in decrease of basal and acute vascular permeability[40, 41]. In other aspect, our study showed that neutrophil CEACAM1 expression was positively correlated with plasma IL-10 level in patients at IS acute and subacute phases. Previous *in vitro* and *in vivo* models of IS showed IL-10-mediated neuroprotection directly and indirectly[19], but the specific mechanism remained unclear. Related studies are needed to further illustrate the exact mechanism in the future.

Neutrophils, one of the main innate immunoreactive cells, play crucial roles in killing the bacteria and fungi. In a previous study, CEACAM1 was considered to be related to the proliferation of neutrophils and granulopoiesis[28], as well as the secretion and apoptosis of cytokines[27, 30]. Our data showed that the CEACAM1 on the surface of neutrophils in the IS patients was significantly higher compared to the normal control. Nevertheless, our data showed that there were no statistical differences in the apoptosis of CEACAM1 positive neutrophils compared with negative counterparts. On this basis, we sepculated that there might be other molecules involving in regulation of CEACAM1-induced neutrophil proliferation and granulopoiesis.

CEACAM1 on the surface of neutrophils in the peripheral blood is an important source of plasma CEACAM1[42]. Our data indicated that plasma CEACAM1 was positively correlated with the CEACAM1 on the neutrophils in the peripheral blood of subacute stage stoke patients. To our best knowledge, Tim-3, serving as an important immune checkpoint factor of T lymphocytes, is mainly responsible for the negative regulation of T lymphocytes[43]. As a ligand of Tim-3, CEACAM1 could mediate the function of Tim-3 modulated T lymphocytes[44, 45]. Thus, it could affect the Tim-3 positive T lymphocyte proliferation[29], function[46] and secretion of cytokines[31]. Our previous study indicated that there was elevation of Tim-3 on the peripheral blood T lymphocytes in the stroke[32]. We speculated that CEACAM1 could bind with Tim-3 through the T lymphocyte surface, which may induce injury of immune function of lymphocytes at the late stage IS patients, and finally result in increased risk of infection.

Recent study indicated that stroke patients with increased plasma NGAL showed poorer prognosis[47]. Our data on the IS patients were in line with the study by Hochmeister Sonja et al[47], in which significant increase was noticed in the plasma NGAL at the subacute stage. In a previous study, the Cys-87 in NGAL could form a disulphide bond with an as yet unidentified cysteine residue in the PEX domain of MMP-9[34]. Evidences indicated that NGAL, MMP-9/NGAL and MMP-9 were co-expressed in the activated monocytes and neutrophils. NGAL could regulate the activity of MMP-9. In the presence of NGAL, the degradation of MMP-9 was obviously inhibited, which led to the preserve of the MMP-9 activity[35]. In this study, despite the fact that MMP-9 elevation was merely noticed at the subacute stage, there was persistent elevation of plasma NGAL. We speculated from our data that high concentration of NGAL may activate or preserve the MMP-9 activity. This may contribute to the fact that the activity level of MMP-9

was higher than the normal level even its concentration was not higher in the stable period. On this basis, we speculated that NGAL and MMP-9 played important roles in BBB injury.

In summary, CEACAM1 expression level in neutrophils was higher in stroke patients of different stages than that in normal individuals. Additionally, significant elevation was noticed in the serum CEACAM1, MMP-9 and NGAL in stroke patients. CEACAM1 may serve as an important inhibitory regulator for mediating the early-stage BBB injury induced by MMP-9. Nevertheless, persistent high-expression of serum NGAL would combine with MMP-9, which may contribute to the persistent injury of BBB. Moreover, as a ligand of Tim-3, CEACAM1 may involve in the negative immunoregulation of T lymphocytes, which may lead to severe immune dysfunction and subsequent infections. In the near future, attempts should be made to verify the interaction among CEACAM1, MMP-9 and NGAL, and to further illustrate the roles of CEACAM1 in the stroke, ischemia-reperfusion injury and secondary infection in the patients and animal models.

Conclusions

In this study, rapid elevation of neutrophil CEACAM1 and change of levels of associated inflammatory mediators in the serum were observed at the acute stage of cerebral IS. The results implied that CEACAM1 may serve as an inhibitory regulator affecting the progression of focal cerebral ischemia, especially involved in the BBB injury progress.

Declarations

Ethics approval and consent to participate

All experimental procedures and protocols were approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Consent for publication

Not applicable

Conflict of interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Availability of data and materials

All the data were available upon appropriate request.

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Authors' Contributions

WW carried out the flow cytometric analysis, participated in the design of the study, and helped in drafting the manuscript. YZ determined the relations among CEACAM1, NGAL and MMP-9, participated in the design of the study and revised the manuscript. YW carried out supplemental experiments and revised and polished the manuscript. SS and HR participated in the design of the study, and helped in drafting the manuscript. RZ analyzed and interpreted the results and revised the manuscript. PL participated in the sample collection. QX and ZC conceived the study, participated in its design and coordination, and helped in drafting the manuscript. All authors read and approved the final manuscript. Wei Wu and Yi Zhang contributed equally to this work.

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Figures

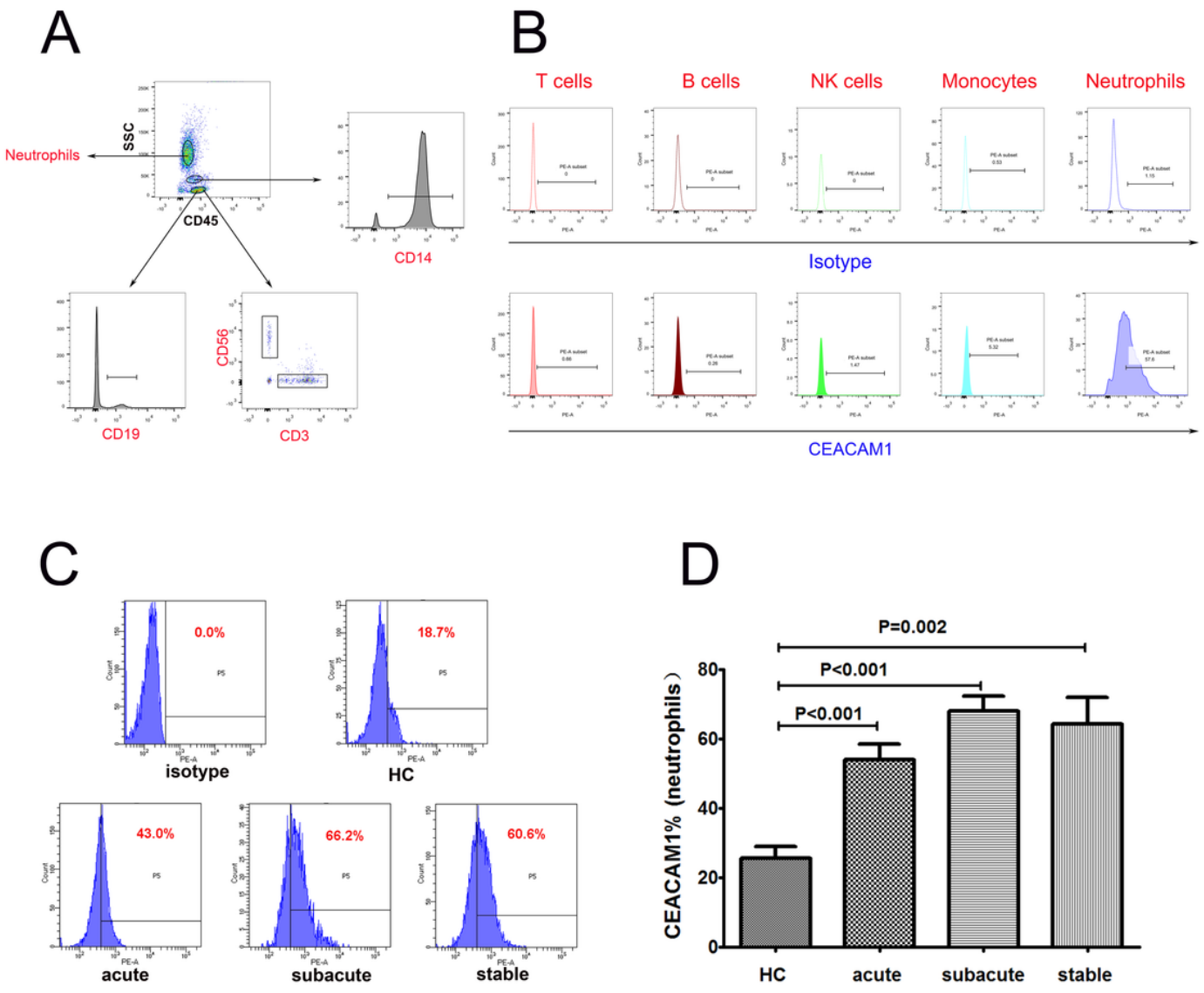

Figure 1

Analysis of CEACAM1 on neutrophils. (A) The T cells (CD3+), B cell (CD19+), NK cells (CD3-CD56+), monocyte (CD14+) and neutrophils were selected from the CD45 positive cells. (B) Expression of CEACAM1 in the IS patients. (C) Flow cytometry gating strategy for the determination of CEACAM1 on neutrophils. (D) Comparison of percentage of CEACAM1 positive cells in peripheral neutrophils in the IS patients and normal individuals. A post hoc test was utilized for the analysis.

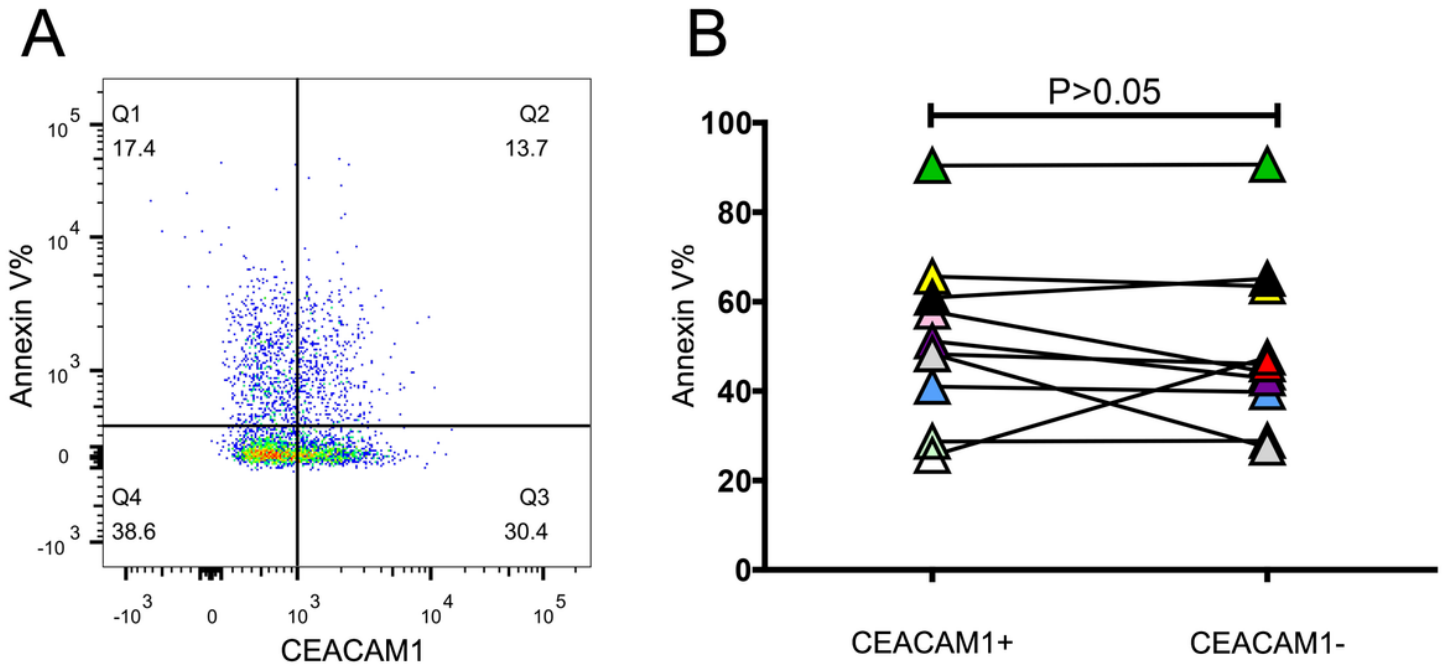

Figure 2

Expression of Annexin V in the CEACAM1 positive and negative cells in patients from the subacute group. There was no statistical difference between the CEACAM1 positive and negative cells (n=10). Statistical analysis was performed by the Mann–Whitney test.

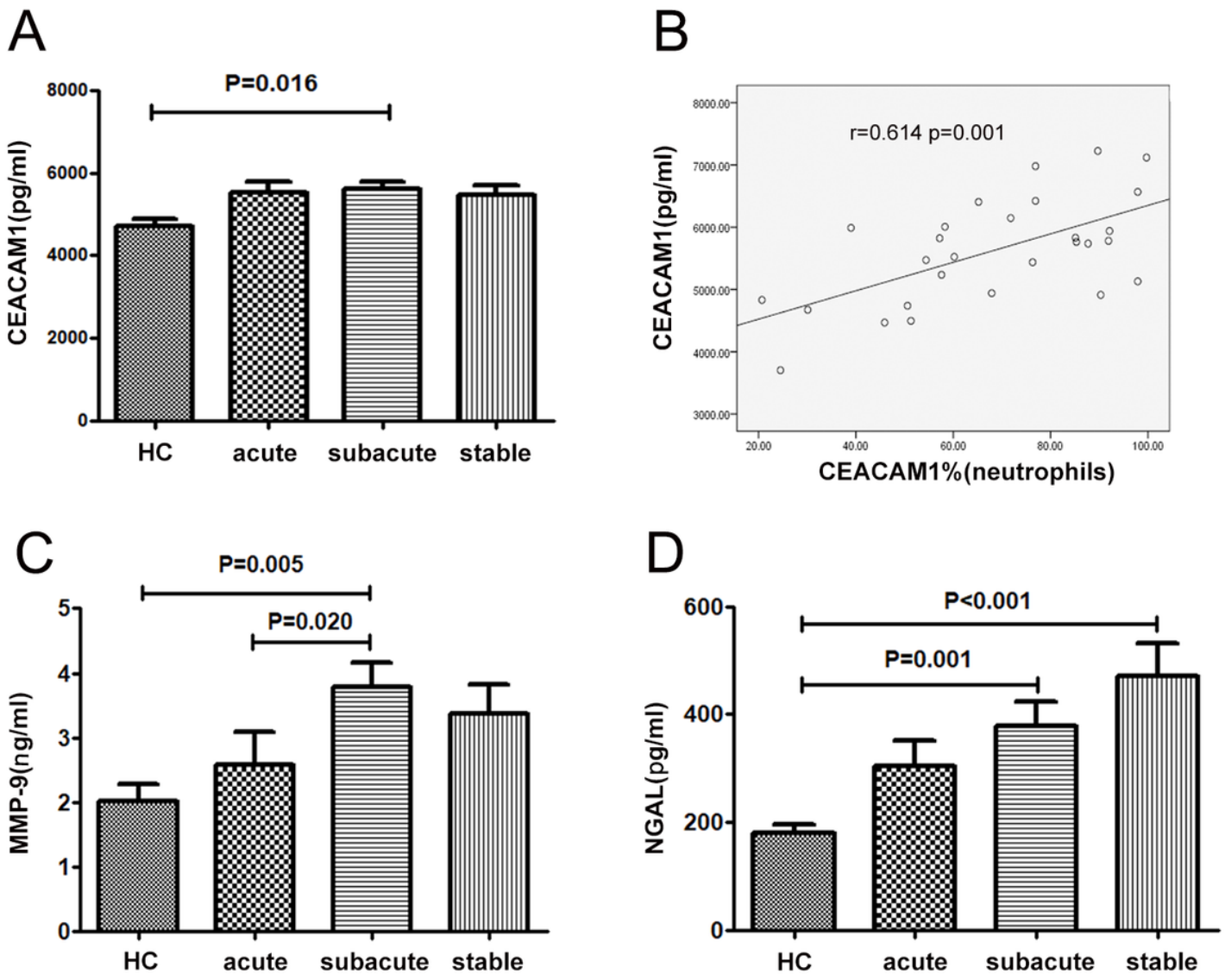

Figure 3

Content of CEACAM1, NGAL and MMP-9 in the plasma of patients with IS. (A) Comparison of plasma CEACAM1 concentration in the IS patients and normal control. (B) Correlation analysis between plasma CEACAM1 content and percentage of CEACAM1 positive neutrophils in the subacute group. (C, D) Comparison of plasma MMP-9 and NGAL in the IS group and the normal control group. A post hoc test or Kruskal-Wallis was utilized for the analysis.

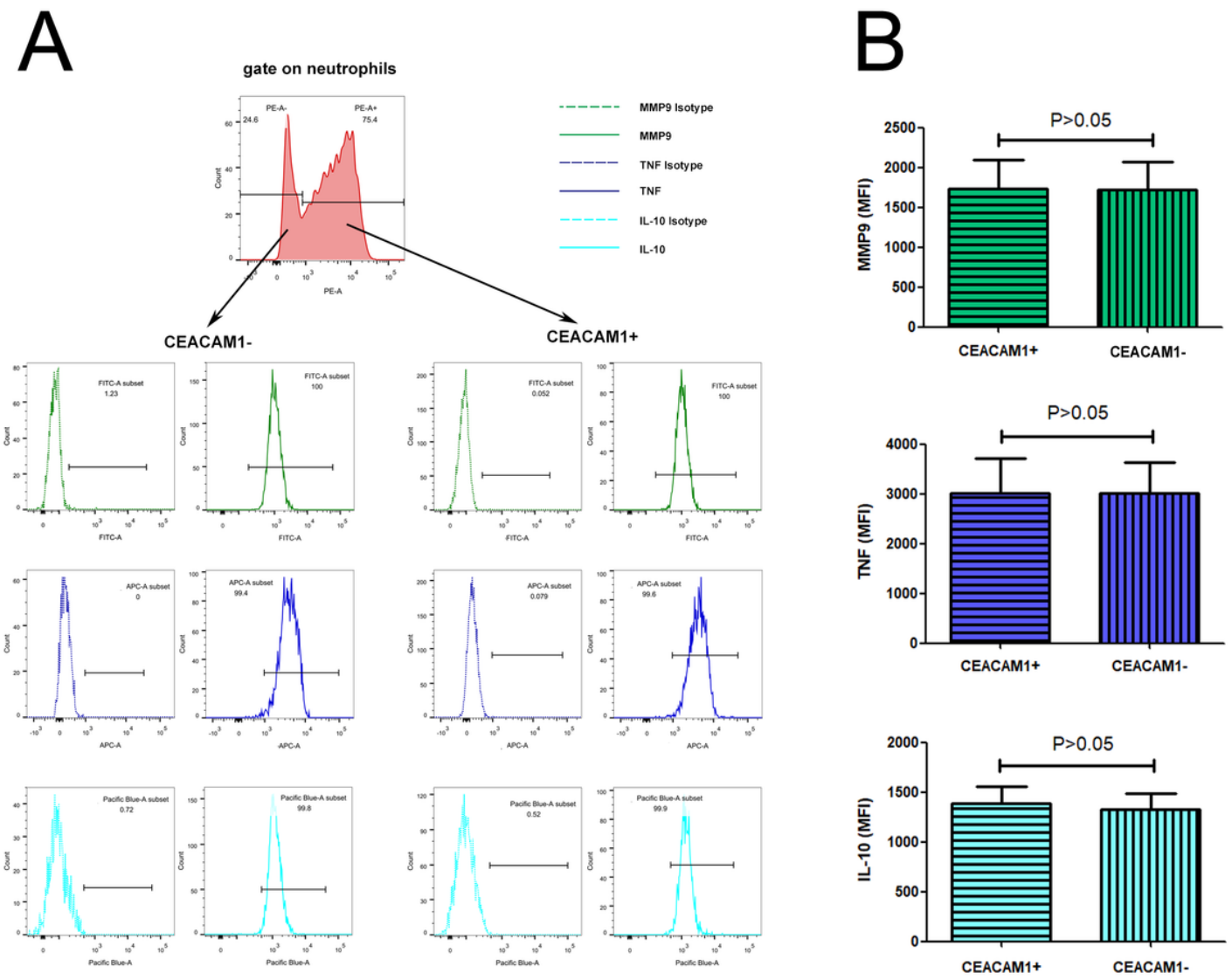

Figure 4

Expression of MMP-9, TNF-α and IL-10 in CEACAM1 positive and negative neutrophils in patients at subacute stage after treating with LPS (100ng/ml) for 2 h. (A) Gating strategy. The dotted line represented the sample isotype, and the solid line represented the measurement value. (B). MFI showed no statistical differences in the MMP-9, TNF-α and IL-10 in the CEACAM1 positive and negative neutrophils (n=5). Statistical analysis was performed by the Mann–Whitney test.

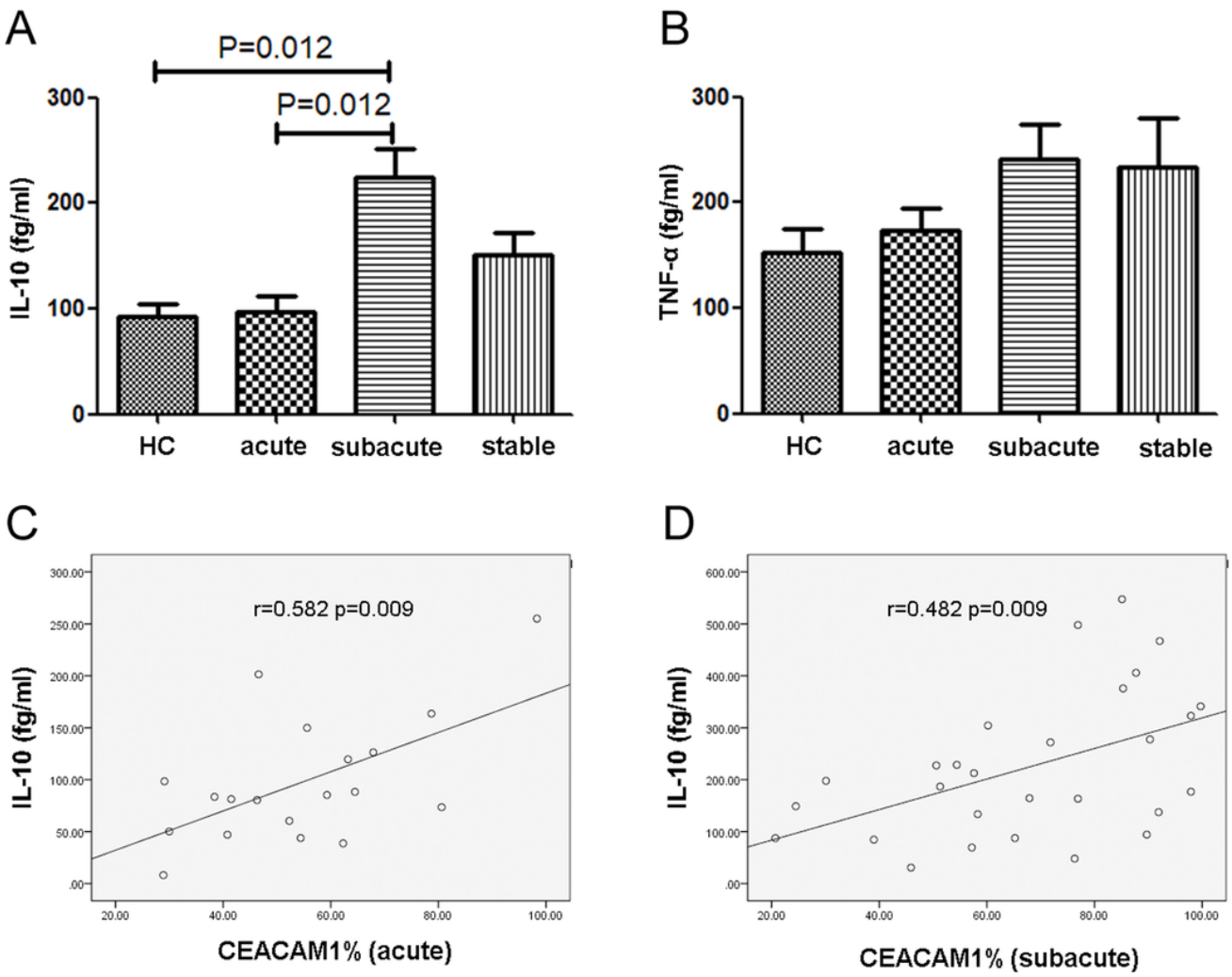

Figure 5

Content of IL-10 and TNF in the plasma of patients with IS. (A) Comparison of plasma IL-10 concentration in the IS patients and normal control. (B) Comparison of plasma TNF concentration in the IS patients and normal control. (C) Correlation analysis between plasma IL-10 content and CEACAM1 positive neutrophils in the acute group. (D) Correlation analysis between plasma IL-10 content and CEACAM1 positive neutrophils in the subacute group. Correlation determined by Pearson correlation analysis.

Supplementary Files

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- SupplementaryFigure1.tif