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Monocytes Are Major Players in the Prognosis and Risk of Infection After Acute Stroke

Xabier Urra, MD; Álvaro Cervera, MD, PhD; Víctor Obach, MD; Núria Climent, PhD;
Anna M. Planas, PhD; Ángel Chamorro, MD, PhD

Background and Purpose—Monocytes participate in adaptive and innate immune responses. Monocyte numbers increase in patients with stroke associated infection (SAI) or severe stroke. Whether changes in monocytes are related to specific effects, or simply mark brain damage, remains unsettled.

Methods—We used flow cytometry in 45 consecutive strokes and 12 healthy controls to assess the time course of monocytes, their phenotype, and the production of cytokines after stimulation. Cortisol, TNF-α, IFN-γ, and IL-10 were measured in serum and metanephrine in plasma. The effects of humoral and cellular parameters on the risk of SAI and poor outcome were tested in multivariate analyses adjusted for confounders (NIHSS score, age, and tube feeding).

Results—Surface expression of human leukocyte antigen-DR, Toll-like receptor-2, and production of TNF-α in monocytes were independently associated with stroke. Distinct immune mechanisms were related with functional outcome and the risk of SAI; the signature of SAI included an increase of cortisol, metanephrine, and IL-10 in serum, and reduced production of TNF-α in monocytes; poor outcome was associated with increased expression of Toll-like receptor-4 in monocytes (OR, 9.61; 95% CI, 1.27–72.47). SAI did not predict poor outcome (OR, 5.63; 95% CI, 0.45–70.42; $P=0.18$).

Conclusions—In human stroke, poor outcome is associated to innate responses mediated by Toll-like receptor-4 in monocytes. SAI may result from the immunosuppressive and antiinflammatory effects of corticoids, catecholamines, IL-10, and deactivated monocytes. Early treated SAI does not contribute significantly to additional brain damage. These findings encourage the exploration of strategies aimed to inhibit Toll-like receptor-4 signaling in acute stroke. (***Stroke***. 2009;40:1262-1268.)

Key Words: acute stroke ■ flow cytometry ■ immunology ■ monocytes ■ stroke-associated infection

The relevance of immune mechanisms in patients with acute stroke is increasingly recognized.[1–4] Ischemic and hemorrhagic stroke disrupt the blood–brain barrier, damage brain cells, and allow self antigens from the central nervous system to interact with circulating and resident immune cells.[5] The exchange of immunologic signals from and to the brain is facilitated by a rich bidirectional regulatory network between the central nervous system and the adaptive and innate immune systems. This network includes neural pathways that innervate the lymphoid organs,[6] neuroendocrine glands,[7] and humoral messengers such as cytokines, adrenomedullary hormones, or glucocorticoids.[8] For many years these neural, humoral, and cellular pathways have been considered essential in the physiological regulation of the immune system, but until recently they had not received attention in the setting of acute stroke.

Pioneer studies in rats described the arrival of circulating monocytes to capillaries and venules of brain ischemic areas as early as 4 hours after stroke onset, but their specific effects were not further elucidated.[9] Monocytes are of paramount immunologic relevance because they contribute to adaptive immunity as antigen-presenting cells, and they are the main effectors of innate immunity through the expression of pattern recognition receptors.[10,11] These receptors include the Toll-like receptors (TLR), which are linked to intracellular signal transduction pathways that regulate the inflammatory response.[12] There are at least 10 distinct TLR families in humans, and TLR2 and TLR4 are the best-studied in the central nervous system.[13]

Monocytes express human leukocyte antigen-DR (HLA-DR) to bind to foreign and self peptides, which are then recognized by CD4$^+$ T cells that secrete cytokines that amplify the immune response.[14] Other relevant molecules in monocytes are CD49d, which interacts with vascular cell adhesion molecule-1 and allows monocytes and lymphocytes to cross the endothelial wall to gain access to tissues,[15] and

CD86, a costimulatory molecule that is expressed on activated antigen presenting cells, especially when TLR are stimulated.[16] However, the specific role of monocytes in patients with acute stroke and the overall harms and benefits of inflammatory and immune responses remain incompletely understood.

We posit that the study of infections complicating the course of acute stroke could serve as a useful clinical model to address the relevance of immune responses after stroke. In experimental studies, stroke-associated infection (SAI) may result from a state of stress-mediated reduced immune competence that is associated with increased mortality.[17] At the bedside, SAI occurs most frequently in patients with severe stroke and reduced immune competence.[4,18] However, it is also arguable that reduced immune competence could be beneficial after stroke because it would limit the inflammatory response to brain injury. Clarification of this conundrum is required to judge the need of immunomodulatory therapies in acute stroke, and to define specific immune targets that might translate into a better risk–benefit profile. This study supports the potential value of immunomodulation after stroke and suggests that the innate proinflammatory responses mediated by monocytes are promising therapeutic targets.

Subjects and Methods

Subjects

We studied 45 consecutive stroke patients with a prestroke modified Rankin Scale score ≤2, and a National Institutes of Health Stroke Scale (NIHSS) score on admission >3. Patients were first evaluated at a median of 180 minutes (interquartile range, 120–350) of stroke onset. Exclusion criteria included a history of infection or the use of antibiotics, immunosuppressants, or steroids within the preceding 3 months. Although the high prevalence of infections before stroke increases the possibility that an infection diagnosed after admission was present before, we tried to avoid the inclusion of those patients by carefully looking for signs and symptoms of infection in the interview, the first physical examination, and the emergency tests. The study was approved by the local ethics committee and all participants or their legal representatives signed a written informed consent. Patients had a brain CT scan or MRI on admission and were managed in the Stroke Unit according to European Guidelines.[19] Neurological impairment was assessed daily using the NIHSS until day 7, and at day 90. Functional outcome was assessed with the modified Rankin Scale and NIHSS score at day 90. Favorable outcome was defined as a modified Rankin Scale of 0 to 2 and a NIHSS of 0 or 1. According to the ESPIAS trial,[20] SAI was defined as a body temperature >37.8°C in patients with suggestive symptoms (ie, cough, dyspnea, pleuritic pain, urinary tract symptoms), or white blood cell count >11 000/mL or <4000/mL, pulmonary infiltrate on chest x-rays, or cultures positive for a pathogen during the first week after stroke. SAI was first assessed by stroke neurologists and then validated by specialists on infectious diseases external to the current investigation. Treatment with appropriate antibiotics was started whenever SAI was suspected, and intravenous ceftriaxone was the starting regime unless contraindicated. Twelve relatives of the patients, all free of symptomatic central nervous system disorders, served as controls.

Flow Cytometry

Blood samples were collected at a median delay of 180 minutes after stroke onset before any medication was started, and between 8:30AM and 9:00AM at days 2, 7, and 90 after admission. The phenotype of monocytes was analyzed immediately after blood extraction by investigators blinded to clinical end points. The following monoclonal antibodies were used: TLR2 and IgG1 isotype control conjugated

to fluorescein isothiocyanate, TLR4, CD49d (very late antigen-4), CD86 (B7–2), HLA-DR, and IgG1 isotype control conjugated to phycoerythrin, CD45 conjugated to Peridinin-chlorophyll-protein, and CD14 and IgG1 isotype control conjugated to allophycocyanin (all from Pharmingen, except for TLR2 and TLR4, which were from Serotec). Monoclonal antibodies were mixed with the cell suspension and incubated for 15 minutes at room temperature in the dark. After erythrocyte lysis and 2 washes, acquisition was performed on a FACSCalibur flow cytometer (BD Biosciences). CellQuest software (BD Biosciences) was used for analysis. Surface molecule expression was quantified by converting median fluorescent intensity values into molecules of equivalent soluble fluorochrome units using standardized fluorescent beads (Quantum fluorescein isothiocyanate and Quantum phycoerythrin Medium Level; BangsLabs). Molecules of equivalent soluble fluorochrome units were obtained after substraction of the isotype control molecules of equivalent soluble fluorochrome units.

Data of the same study population focused on the effects of stroke on the adaptive immune system have been reported elsewhere.[21]

Intracellular Cytokines

After diluting 1000 μL of whole blood 1:1.5 in RPMI 1640 (GIBCO BRL; Breda), it was stimulated for 4 hours with 1 μg/mL lipopolysaccharide in the presence of 10 μg/mL Brefeldin A (both from Sigma). Incubation and intracellular staining was performed according to the manufacturer's protocol (BD Biosciences) using fluorescein isothiocyanate conjugated anti-TNF-α and phycoerythrin conjugated anti-IL-10 antibodies and their respective controls. Monocytes were recognized by their staining with CD45 and CD14, and the results were expressed as the proportion of TNF-α or IL-10–positive monocytes.

Cortisol, Metanephrine, and Cytokines

Between 8:30AM and 9:00AM of day 1, serum cortisol levels were measured using an enzyme immunoassay, and unconjugated levels of metanephrine (MN) were measured in plasma by competitive enzymatic immunoassay, as previously reported.[21] IL-10, TNF-α, and IFN-γ levels were determined in serum at days 0, 2, 7, and 90 using a BD Cytometric Bead Array Human Th1/Th2 cytokine kit (BD Biosciences) according to the manufacturer's protocol.

Statistical Analysis

Differences in patients and between patients and controls were calculated with the Student t test or Mann–Whitney U test as appropriate. Correlations were calculated with the Spearman Rank correlation coefficient. The last observation carried forward method was used for missing clinical values. SAI and outcome were dependent variables assessed in logistic regression models adjusted for baseline stroke severity (NIHSS score), age, and tube feeding.[18] The area under a receiver-operator characteristic curve was used to compare the ability of different logistic regression models to predict outcome. All tests were performed using the SPSS software version 14.0 (SPSS Inc). $P<0.05$ were considered statistically significant.

Results

Stroke-Associated Infection

The main traits of the study population are shown in Table 1. Patients with ischemic stroke were older than those with hemorrhagic stroke (76.6 years; SD, 9.0 vs 65.2 years; SD, 13.9; $P=0.003$), but the stroke subtype resulted in no significant differences in risk factors, clinical findings, and laboratory results (data not shown). Exploratory analyses by ischemic subtype or hemorrhage location were not performed. SAI occurred in 14 (31%) patients at a mean delay of 2.5 days after symptom onset and included pneumonia (n=5), tracheobronchitis (n=5), urinary tract infection (n=2), and other infections (n=2). Expectedly, SAI prevailed in patients

Table 1. Main Characteristics of the Study Population

	Controls	All Patients	SAI	Poor Outcome
N	12	45	14	33
Demographics, risk factors				
Age, yr, mean (SD)	73.7 (10.7)	73.8 (11.4)	74.6 (14.6)	75.5 (11.9)
Male, %	50	56	79	54
Smokers, %	0	10	14	13
Hypertension, %	45	76	71	73
Diabetes mellitus, %	0	32*	43	30
Dyslipidemia, %	18	34	36	37
Coronary artery disease, %	9	15	0	10
Peripheral arterial disease, %	0	10	14	10
Previous stroke, %	0	15	7	13
Tube feeding, %		27	57††	40‡
Urinary catheters, %		27	43	37
Antibiotics within 1 wk, %		42	100††	57‡
rtPA, %		38	57	39
Qualifying stroke subtype, %				
Ischemic		76	79	82
Hemorrhagic		24	21	18
Baseline parameters, mean (SD)				
SBP, mm Hg	149 (25)	165 (28)	170 (30)	168 (28)
DBP, mm Hg	77 (15)	77 (18)	79 (21)	81 (15)
Temperature	36 (0.5)	36.1 (0.6)	36 (0.5)	36.1 (0.6)
Neurological course, NIHSS score, median (IQR)				
Baseline		12 (6.5–18)	19 (13.2–20.2)††	14 (7.5–19)‡
48 hr		12 (5–17.7)	18 (14–20)†††	15.5 (9–19)‡‡‡
Day 7		8 (3.5–17.5)	18 (13.7–27.7)†††	14 (6–20)‡‡‡
Day 90		4 (1–14.7)	15.5 (4–42)††	6 (3–42)‡‡‡
Death at day 90, %		20	67††	
Neurohormonal response				
Cortisol, μg/dL, mean (SD)	18.5 (6.7)	23.9 (7.1)*	28.8 (8)††	24.8 (6.8)
Metanephrine, pg/mL, mean (SD)	15.9 (17.5)	16.6 (14.1)	21.2 (10.7)††	16.2 (10.5)

*Patients vs controls.
†SAI vs no SAI.
‡Poor vs favorable outcome.
1 symbol=$P<0.05$; 2 symbols=$P<0.01$; 3 symbols=$P<0.001$.

fed by nasogastric tube and in those with poor outcome (Table 1), but SAI was not independently associated with poor outcome in models adjusted for stroke severity, age, and tube feeding (OR, 5.63; 95% CI, 0.45–70.42; $P=0.18$).

Cortisol and Metanephrine

Cortisol levels were higher in patients than in controls, highest in patients with SAI, although unrelated to clinical outcome (Table 1). MN levels were similar in patients and controls, highest in patients with SAI, and unrelated to clinical outcome (Table 1). Cortisol was correlated with MN ($r=0.39$; $P=0.02$) and baseline NIHSS score ($r=0.38$; $P=0.02$). In adjusted models, SAI increased with higher levels of cortisol (quartiles: OR, 2.51; 95% CI, 1.08–5.84; $P=0.03$) and MN (quartiles: OR, 3.00; 95% CI, 1.23–7.32; $P=0.01$).

Serum Levels of Cytokines

As shown in Table 2, patients had higher IFN-γ, lower TNF-α, and similar IL-10 levels than controls. In multivariate analysis, SAI was associated with increased IL-10 at baseline, quartiles (OR, 4.56; 95% CI, 1.41–14.77; $P=0.01$), and at day 2 (OR, 2.80; 95% CI, 1.08–7.32; $P=0.03$). Levels of TNF-α and IFN-γ were unrelated to SAI, and poor outcome was unrelated to the time course of IFN-γ, IL-10, or TNF-α (Table 2).

Monocytes After Stroke: Phenotype and Cytokine Production

The number of monocytes increased in patients compared with controls, as shown in Figure 1A. Patients also had significantly lower expression of HLA-DR (Figure 1B), and enhanced expression of TLR2 (Figure 1C). The expression of

Table 2. Cytokines in Serum in Patients and Controls and Their Relation With SAI and Poor Outcome at 3 Months in Models Adjusted for Age, Baseline NIHSS Score, and Tube Feeding

	Controls	All Patients	SAI	Poor Outcome
TNF-α pg/mL, mean (SD)				
Baseline	3.40 (1.89)	2.21 (1.84)	1.80 (1.19)	2.38 (2.04)
48 hr		2.18 (2.52)	1.74 (1.46)	2.37 (2.75)
Day 7		2.35 (2.58)	1.98 (1.39)	2.05 (1.10)
Day 90		2.81 (1.55)	2.56 (1.64)	3.21 (1.67)
IFN-γ pg/mL, median (IQR)				
Baseline	3.05 (1.20–3.66)	4.12 (1.45–6.76)	4.27 (2.46–6.27)	4.24 (2.33–6.46)
48 hr		3.69 (2.22–5.18)	3.45 (1.1–5.26)	3.61 (2.2–5.18)
Day 7		3.78 (2.65–5.57)	3.37 (1.82–4.99)	3.78 (2.14–5.46)
Day 90		4.48 (2.83–6.45)*	4.14 (2.43–4.76)	4.25 (2.71–6.14)
IL-10 pg/mL, median (IQR)				
Baseline	1.58 (0.01–1.81)	1.62 (1.21–3.01)	3.01 (1.67–5.38)†	1.67 (1.29–3.53)
48 hr		1.64 (1.39–2.14)	2.13 (1.55–4.90)†	1.68 (1.38–2.14)
Day 7		1.53 (1.25–2.11)	2.16 (1.98–5.11)	1.67 (1.24–2.17)
Day 90		1.69 (1.42–2.25)	1.54 (1.27–2.13)	1.64 (1.34–2.18)

*$P<0.05$ patients vs controls.

†$P<0.05$ SAI vs no SAI.

TLR4, CD86, and CD49d did not differ between patients and controls (Figure 1D to 1F). Stroke patients had significantly lower proportion of TNF-α producing monocytes after stroke, whereas the production of IL-10 was similar than in controls, as shown in Figure 2. In logistic regression adjusted for age and risk factors (hypertension and diabetes), at day 2, stroke was associated with the magnitude of the surface expression of HLA-DR (OR, 0.30; 95% CI, 0.11–0.79; $P=0.01$) and TLR2 (OR, 3.36; 95% CI, 1.25–9.01; $P=0.02$), and the proportion of TNF-α–producing monocytes (OR, 0.21; 95% CI, 0.06–0.68; $P=0.01$).

Monocytes and Stroke-Associated Infection

SAI was associated in univariate analyses with increased number of monocytes on day 2 ($P=0.02$) to at least day 7 ($P=0.01$), reduced expression of HLA-DR, CD86 and CD49d (Figure 3A-C), and lower proportion of TNF-α–producing monocytes (Figure 3D). Lower TNF-α production on day 2 (quartiles) remained associated to SAI in adjusted models (OR, 0.27; 95% CI, 0.09–0.80; $P=0.01$). In exploratory analyses, monocyte deactivation was correlated with the levels of cortisol ($r=-0.40$; $P<0.05$), and IFN-γ on admis-
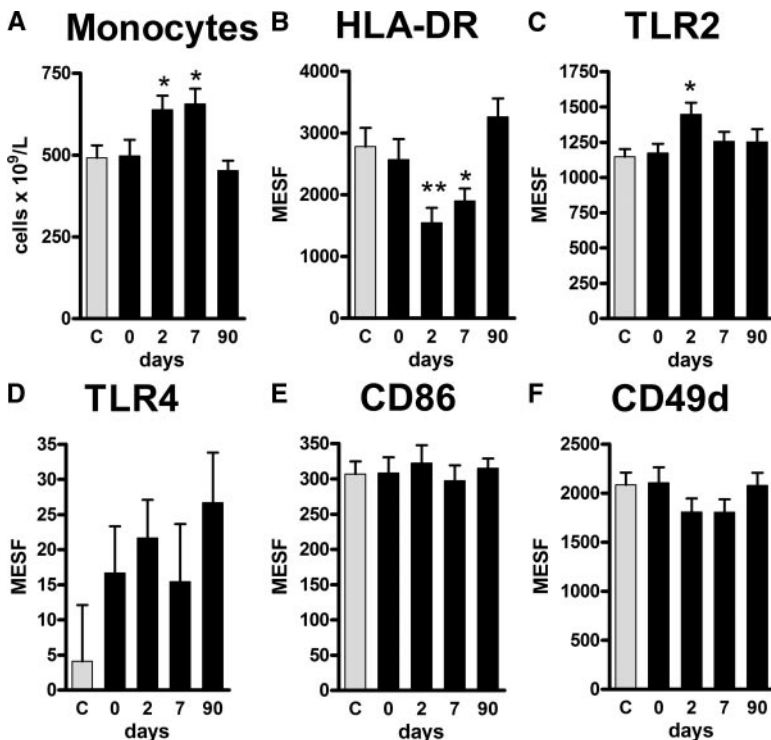

Figure 1. Time course and phenotype of circulating monocytes in acute stroke. Patients had increased numbers of circulating monocytes (A), decreased expression of HLA-DR (B), and increased expression of TLR2 (C). The surface expression of TLR4, CD86, and CD49d did not significantly differ between controls and patients (D–F). Values are mean±SEM. *$P<0.05$, ** $P<0.01$, control vs stroke; C indicates controls; n(controls)=13, n(day 0)=37, n(day 2)=39, n(day 7)=41, n(day 90)=33. MESF indicates molecules of equivalent soluble fluorochrome.
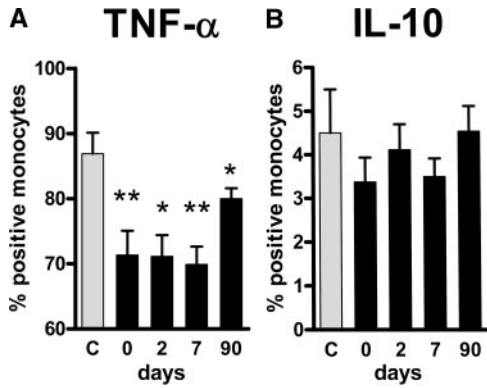
Figure 2. Cytokine production in monocytes after stimulation with lipopolysaccharide in acute stroke. Stroke patients had a significant decrease in the proportion of TNF-α producing monocytes, especially during the acute phase (A), whereas the production of IL-10 was similar to that in controls (B). Values are mean±SEM. *$P<0.05$, ** $P<0.01$, control vs stroke; n(controls)=11, n(day 0)=20, n(day 2)=36, n(day 7)=27, n(day 90)=32.

sion ($r=-0.44$; $P=0.01$). Surface expression of TLR2 and TLR4 was unrelated to SAI (data not shown).

Monocytes and Stroke Outcome

Poor outcome was associated with decreased expression of HLA-DR at baseline ($P=0.06$), on day 2 ($P=0.03$), day 7 ($P=0.01$), but not on day 90 after stroke ($P=0.23$); however, these findings were not significant in adjusted models. As shown in Figure 4, surface expression of TLR4 at day 2 increased in patients with poor outcome ($P=0.03$), and the effect remained significant in adjusted models (quartiles: OR, 9.61; 95% CI, 1.27–72.47; $P=0.02$). Using receiver-operator characteristic curve analysis, adding TLR4 expression to clinical variables increased the capacity to predict poor outcome (area under the curve) from 0.73 to 0.95. Expression of TLR2 and other receptors in monocytes and cytokine
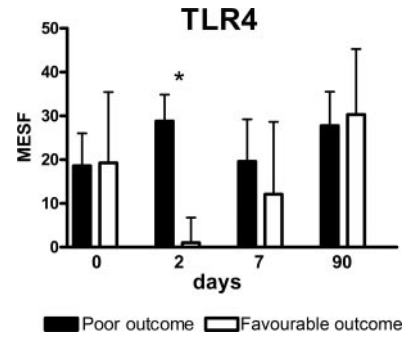

Figure 4. Time course of TLR4 expression and outcome after stroke. Higher expression of TLR4 on monocytes at day 2 was associated with worse outcome. This association remained significant after adjusting for potential confounders. Values are mean±SEM. *$P<0.05$, favorable vs poor outcome; n(day 0)=37, n(day 2)=39, n(day 7)=41, n(day 90)=33. MESF indicates molecules of equivalent soluble fluorochrome.

production was not associated with outcome (data not shown).

Discussion

The clinical relevance of immune responses after acute stroke is stressed in this study, which showed that distinct mechanisms are associated with clinical outcome and the risk of SAI. Previously, the most consistently reported clinical predictors of SAI included being older, greater baseline stroke severity, total anterior cerebral infarction, and dysphagia.[23] In this study, we confirmed these data and the association between SAI and several cellular and humoral markers including a decreased capacity to release TNF-α in stimulated monocytes, increased levels of MN,[22] and increased IL-10 in serum.[24] The study first reported the independent association between higher levels of cortisol and increased risk of SAI. In addition, the study confirmed that monocytes in patients with
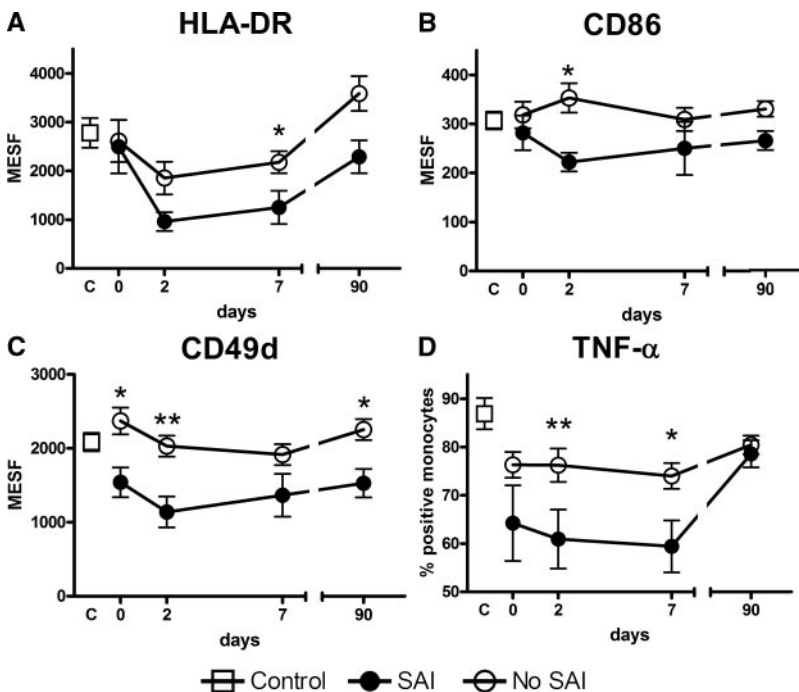

Figure 3. Time course and phenotype of circulating monocytes in relation to SAI. Patients with SAI had lower expression of HLA-DR (A), CD86 (B), and CD49d (C), as well as lower proportion of TNF-α producing monocytes (D). Values are mean±SEM. *$P<0.05$, SAI vs no SAI. A–C, n(controls)=13, n(day 0)=37, n(day 2)=39, n(day 7)=41, n(day 90)=33. D, n(controls)=11, n(day 0)=20, n(day 2)=36, n(day 7)=27, n(day 90)=32. MESF indicates molecules of equivalent soluble fluorochrome.

acute stroke show a reduced expression of HLA-DR and decreased capacity to release TNF-α after stimulation.[25,26] Yet the study added new insights in brain–immune interactions, because it showed for the first time to our knowledge that stroke patients also had an increased expression of TLR2 in monocytes, and evidenced that the expression of TLR4 is an independent predictor of functional outcome.

The independent association between increased expression of TLR4 in monocytes and poor outcome after stroke in humans is consistent with previous experimental data indicating that TLR4-deficient mice had smaller infarctions and less inflammatory response after an ischemic insult,[27] and that the brain damage caused by stroke prime mechanisms that signal through TLR4.[28] We recently reported[21] an association between poor outcome after stroke and increased expression of CD86 in B lymphocytes. Because the expression of CD86 is increased if TLRs are stimulated,[16] this association might be the result of an increased innate response elicited by TLR4 signaling. The relationships between TLR4 expression and poor clinical outcome but not SAI reinforce the concept that TLR4 can be activated by endogenous ligands without the intervention of exogenous pathogens.[29] The expression of TLR2 was increased on day 2 in patients with stroke, although SAI and poor outcome were not associated with TLR2 in adjusted models. This increased expression of TLR2 in acute stroke agrees with previous studies demonstrating that TLR2 signaling is involved in the induction of inflammatory and tissue-repair genes after tissue injury.[30]

A decreased capacity to release TNF-α in stimulated monocytes has been previously described in patients with acute stroke,[25,26] and in patients with SAI.[25] We confirmed a reduced capacity of monocytes to produce TNF-α but not IL-10 in stroke, and a greater reduction in patients with SAI. Monocyte deactivation was detected on admission (median, 3 hours); it was severest on day 2, and similar to controls on day 90. The relevance of monocyte deactivation observed in patients fed by nasogastric tube suggests a potential mechanism to explain the high incidence of pneumonia in these patients.[31] The study also confirmed the association between hypercortisolemia and monocyte deactivation,[32] but further studies will be required to unravel the molecular mechanisms that limit the inflammatory drive of monocytes.

Several studies have described an increment of cortisol levels after stroke, particularly in patients with severe stroke.[33–36] However, it had not been addressed whether increased cortisol favor the incidence of SAI, as we first report in this study. Our current findings are in accord with the overall immunosuppressive effects of glucocorticoids during stressful conditions,[37] and the simultaneous increase of cortisol and MN support a synchronous hyperactivity of the hypothalamic–pituitary–adrenal axis and the adrenomedullary gland after stroke. At variance with some studies, this neuroendocrine response was not associated with poor outcome[33] or with an exaggerated inflammatory response mediated by cytokines.[38] After adjustment for prognostic confounders, the neurohormonal response was a marker of ongoing stroke severity rather than a contributor to additional injury.

In keeping with previous reports,[24] IL-10 was significantly increased in patients with SAI, and the study stressed a

significant elevation 3 hours after stroke onset, and until at least day 7. Then, assessment of monocyte deactivation and IL-10 in serum could emerge as valuable prognostic aids at the bedside to anticipate very early the risk of SAI. Reduced expressions of surface receptors CD49d, CD86, and HLA-DR were also found in patients with SAI, although not significantly in adjusted models, suggesting that their expression in monocytes was influenced by the extent of tissue damage.

In accordance with current European Guidelines,[39] the study primed the early detection and treatment of incident infections over preventive antibiotic therapy, which has recently shown conflicting results in randomized controlled trials.[20,40,41] Strict adherence to these recommendations resulted in a lack of association between SAI and poor outcome.

This study has several limitations including the relatively small study population and clinical heterogeneity. Subgroup analyses by ischemic stroke subtype or by bleeding location were not performed because large numbers of patients would be required. The study found very similar immunologic results after ischemic and hemorrhagic stroke, in accord with the "danger model" of immune response that proposes that the immune system evolved to primarily recognize danger signals in diseased cells.[42] This interpretation does not exclude the possibility that stroke subtype might influence the interaction between stroke and immune system, but it suggests that the response to brain damage prevails over its specific cause. Also, we acknowledge that greater or different results might have been obtained had control subjects been matched for the burden of atherosclerosis.

Summary

The study showed that different clinical implications derive from the modulation of different surface receptors in monocytes, or their capacity to present antigens or produce inflammatory cytokines. A very rapid switch to an antiinflammatory phenotype in monocytes with a concomitant strong neurohormonal response predisposed to SAI. Stroke outcome depended on innate responses signaled through TLR4 in monocytes, and it was unrelated to SAI. Therefore, our findings encourage further exploration of strategies aimed to inhibit TLR4 signaling in acute stroke.

Disclosures

During the preparation of the manuscript, a work has been published[43] showing an increase in circulating TLR4⁺ monocytes in a group of 19 patients with acute ischemic stroke. Moreover, the expression of TLR4 correlated with stroke severity at 10 days. This work further supports the possibility that innate immune responses signaled through TLR4 influence outcome after stroke in humans.

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