RESEARCH ARTICLE

Plasma cytokine profiling to predict susceptibility to acute mountain sickness

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ABSTRACT. Extensive studies have been performed on acute mountain sickness (AMS), but biomarkers predicting AMS are lacking. Presently, the mainstay methods to identify AMS biomarkers include proteomic and genetic methods at high altitudes or in hypoxic simulated chambers. In the present study, we compared plasma cytokine profiles between AMS-susceptible individuals and AMS-resistant individuals at low altitude by cytokine array analysis. In total, 75 differentially expressed cytokines were identified between AMS-susceptible individuals and AMS-resistant individuals, most involved in inflammation. A quantifiable human custom cytokine antibody array was then used to further test results of cytokine array analysis. Compared to AMS-resistant individuals, the level of insulin-like growth factor binding protein 6 (IGFBP-6) was significantly lower in AMS-susceptible individuals (37,318.99 ± 23,493.11 pg/mL and 25,665.38 ± 25,691.29 pg/mL, respectively; P = 0.04). Conversely, the levels of serum amyloid A1 (SAA1), dickkopf WNT signaling pathway inhibitor 4 (Dkk4), and interleukin 17 receptor A (IL-17RA) were significantly higher in AMS-susceptible individuals than in AMS-resistant individuals (SAA1: 4,069.69 ± 2,502.93 pg/mL vs. 2,994.98 ± 2,295.91 pg/mL, P = 0.05; Dkk4: 2,090.00 ± 2,094.89 pg/mL vs. 1,049.88 ± 1,690.93 pg/mL, P = 0.07; IL-17RA: 11.52 ± 8.33 pg/mL vs. 8.67 ± 6.22 pg/mL, P = 0.08). Although further in-depth research is required to examine the possible role of these cytokines in the development of AMS, these four cytokines may be of use in predicting AMS-susceptibility in a low-altitude environment.

Key words: cytokines, biomarkers, acute mountain sickness, nonaltitude environment, Chinese

Millions of individuals are exposed to risky environments causing acute mountain sickness (AMS) annually [1], which usually occur within 6-12 h after rapid ascent to high altitudes above 2500 m [2]. The effective prophylactic medications for AMS are acetazolamide and dexamethasone, which are administrated a few hours before beginning to ascend to a high altitude [2-4]. However, AMS-resistant individuals may suffer unnecessary toxic side effects from chemoprophylaxis [5-7]. Therefore, it is helpful to evaluate the AMS-susceptibility of people who intend to reach high altitudes.

Genetic and proteomic studies showed that certain biomarkers might be involved in AMS development. The levels of these biomarkers are different in AMS-susceptible individuals and AMS-resistant individuals [8, 9]. Recent studies suggested that inflammation and cytokines are closely related to AMS [10]. Therefore, it may be of interest to investigate AMS-resistant associated cytokines to predict AMS. Cytokine array analysis can be used to analyze plasma samples from AMS-susceptible and AMS-resistant individuals in order to identify AMS associated cytokines. In the present study, to predict AMS-susceptibility in the absence of hypobaric hypoxic exposure, we compared plasma cytokine profiles between AMS-susceptible individuals and AMS-resistant individuals at low altitude. Our results suggest that AMS-resistant individuals have a greater capability to control anti-inflammation damage than AMS-susceptible individuals. Subsequently, we used a quantifiable antibody array to test these results. Our results showed that IGFBP-6, SAA1, Dkk4, IL-17R are differentially expressed between AMS-susceptible and AMS-resistant individuals. These four cytokines may be implicated in predicting AMS-susceptibility in low-altitude conditions.

MATERIALS AND METHODS

Ethics statement

This study has been approved by the Institutional Review Board (IRB) of Lanzhou General Hospital of Lanzhou Military Command (Lanzhou, China). The use of human plasma samples for research purposes was authorized by the IRB of Lanzhou General Hospital of Lanzhou Military Command. All the volunteers agreed to participate in this study by signing a consent document.

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Subjects

In the first stage, we recruited 23 local Chinese volunteers aged 25 to 35 years who primarily resided at an elevation of 400 m or lower in the area of Xi'an, China. In the second stage, we recruited 120 local Chinese volunteers aged 25 to 35 years who primarily resided at an altitude of 1100 m or lower in the area of Tianshui, China. Recruitment, screening, and exclusion criteria used in this study were as described previously [8, 11]. Briefly, screening procedures to assess general health status and to determine eligibility prior to participation included a medical history check, physical and neurological examinations, standard blood and urine analysis, and a maximal exercise test. We excluded those who:

– had any health problems;
– had an abnormal complete blood count, chemistry panel, or liver function results;
– were pregnant or intended to become pregnant in the near future;
– had a history of migraine, headache, seizure, or head injury with loss of consciousness;
– had physical impairments or metal implants that prohibited exercise;
– were smokers;
– had regular prescription medications;
– were unable to reach a workload of least 200 W during the incremental exercise test;
– or had altitude exposure greater than 2500 m within three months of study.

Study design

To identify AMS-susceptible and resistant subjects, the study was conducted in two separate parallel parts. In the first part, cytokine profiles of AMS-susceptible and AMS-resistant subjects were compared by human cytokine antibody array; peripheral venous blood samples were collected, and AMS symptoms were evaluated at Xi'an (altitude of 400 m) 24 hours in advance. To acutely expose volunteers to high altitude, all subjects were transported to Yushu (altitude of 3800 m) from Xi'an within 3 hours by flight. AMS symptoms of subjects were evaluated immediately upon landing (time point: 0h). The following studies were conducted in Yushu Bayi Hospital, which is qualified to conduct phase I trials and can treat high-altitude illness. Two hours after exposure to an altitude of 3800 m, all subjects completed three 5-minute submaximal exercise bouts on a cycle with 15 minutes rest in between in the ward. This was to increase the likelihood of developing AMS [12]. The whole process was monitored by one doctor and two nurses.

In the second part, to analyse levels of cytokines in AMS-susceptible and AMS-resistant individuals, peripheral venous blood samples were collected, and AMS symptoms were evaluated at Tianshui (altitude of 1100 m) 24 hours in advance. To ascend to high altitude rapidly, all subjects were transported to Xidatan (altitude of 4300 m) from Tianshui within 3 days by car. The second part of the study was conducted at 1100 m after participants completed the Lake Louise Questionnaire (LLQ) and peripheral venous blood samples were collected. The subjects drove to the town of Chaka (altitude of 3100 m) within 10 hours and spent the night at an altitude of 3100 m, drove to the mountain of Xitie (altitude of 3500 m) within 8 hours the next day and spent the night at an altitude of 3500 m, and then drove to Xidatan (altitude of 4300 m) within 4 hours on the third day. The whole process was monitored by two doctors and two nurses.

Evaluation of AMS status

In the first part of the study, to evaluate AMS status, subjects completed self-reported sections of the LLQ prior to exposure to 3800 m of high altitude as well as after 3, 6, 9, and 12 hours of hypobaric hypoxia. Similarly, in the second part, subjects completed self-reported sections of the LLQ after 6 h exposure to altitudes of 3100 m, 3500 m, and 4300 m, respectively. Subjects were asked to quantify their degree of headache, gastrointestinal upset, fatigue, and dizziness on a four-point scale (no symptoms : 0, mild : 1, moderate : 2, severe : 3). Subjects with a cumulative LLQ score equal to or greater than 3 with headache after ascent to high altitude within 6 to 12 hours were defined as AMS-resistant individuals. Those with a cumulative LLQ score equal to or less than 2 or without headache after exposure to hypobaric hypoxia were considered AMS-susceptible individuals [13].

In the second part, the group of 23 subjects was ranked according to the LLQ scores after exposure to 3800 m for 6 to 12 hours. The 7 individuals with the highest LLQ scores were assigned to the AMS-susceptible group. The 7 individuals with the lowest LLQ scores were assigned to the AMS-resistant group. Using a similar strategy for the second stage, the group of 120 subjects was ranked after exposure to altitudes of 3100 m, 3500 m, and 4300 m. The 23 individuals with the highest LLQ scores were assigned to the AMS-susceptible group. The 23 individuals with the lowest LLQ scores were assigned to the AMS-resistant group. Although the criteria reduced sample size, it maximized the differences between AMS-susceptible and AMS-resistant individuals, and avoided the inclusion of subjects with ambiguous AMS status [8, 10].

Plasma collection and sample preparation

Plasma collection and sample preparation was performed as described previously [8]. Blood samples were collected in a semi-recumbent position from an antecubital vein by an indwelling intravenous cannula, and then placed in EDTA-coated blood collection tubes. The plasma was separated from blood cells by centrifugation and stored in 0.2 mL aliquots at -80 °C until analysis.

Cytokine array analysis of pooled plasma samples from the AMS-susceptible group and AMS-resistant group

Plasma samples collected from the 7 AMS-susceptible subjects and 7 AMS-resistant subjects were pooled at low altitude (Xi'an). This resulted in two pooled samples for analysis (named AMS-susceptible-LA and AMS-resistant-LA separately). The samples were then centrifuged at 30,000 g at 4 °C, and an aliquot of the supernatant was taken to determine protein concentration by the Bradford protein assay method [14, 15]. The remaining supernatant was kept at -80 °C for further analysis. These two groups of plasma samples (AMS-susceptible-LA and AMS-resistant-LA) from subjects were analyzed using a human cytokine

antibody array (RayBio®, Wayen Biotechnologies, Shanghai, China) which includes 508 cytokines, according to the manufacturer's instructions. Briefly, 100 μL sample (20 μL plasma plus 80 μL 1×PBS, pH = 8) was loaded into a separate dialysis tube. The beaker was placed on a stir plate and dialysed, and buffer gently stirred for at least 3 hours at 4 °C. The 1× PBS buffer was then exchanged and dialysed again for at least 3 hours at 4 °C. Dialyzed samples were labeled by biotin and prepared to be analyzed by cytokine array. Blocking buffer (400 μL per well) was added into each well of the micro-array glass slides for 30 min. Samples (400 μL per well) were added and incubated overnight at 4 °C. The slides were washed in washing buffer and incubated with diluted Streptavidin-Fluor at room temperature for 2 h. Fluorescent signal was detected using an array scanner (Gene Pix 4000B, Axon Instruments, USA) and analyzed using the GenePixPro6.0 (Axon Instruments, USA).

Human custom cytokine antibody array analysis of candidate biomarkers of AMS

Plasma samples collected from the 30 AMS-susceptible subjects (7 from the first stage and 23 from the second stage) and 30 AMS-resistant subjects (7 from the first stage and 23 from the second stage) were pooled at low altitudes (Xi'an and Tianshui). The quantifiable human custom cytokine antibody array was performed by RayBiotech (RayBio®, Wayen Biotechnologies, Shanghai, China) on blinded samples for the following cytokines: AgRP, Dkk-4, EGF, Eotaxin-3, Flt-3 ligand, Follistatin, HCC-4, IGFBP-6, IL-17R, LIGHT, MCP-3, PDGF R beta, Persephin, RAGE, SAA, Siglec-9, TGF-beta3, TPO, VEGF-D, and WIF-1.

Statistics

Data are presented as "mean ± standard deviations" and were analyzed using the "Student's *t* test" where indicated.

RESULTS

AMS status of subjects

In the first part, LLQ scores were lower in the AMS-resistant group than in the AMS-susceptible group at 6 hours (1.00 ± 0.82 *vs.* 3.00 ± 1.00, $P = 0.0015 < 0.05$, $n = 7$), 9 hours (0.43 ± 0.53 *vs.* 6.29 ± 1.80, $P = 2.72\times10^{-6} < 0.05$, $n = 7$), and 12 hours (1.14 ± 1.22 *vs.*4.29 ± 2.06, $P = 0.0046 < 0.05$, $n = 7$) of exposure to an altitude of 3800 m (*table 1*). In the second part, similarly, LLQ scores were lower in the AMS-resistant group than in the AMS-susceptible group at an altitude of 3100 m (0.39 ± 0.50 *vs.* 2.79 ± 1.25, $P = 5.13\times10^{-11} < 0.05$, $n = 23$), altitude of 3500 m (0.13 ± 0.34 *vs.*3.21 ± 1.06, $P = 3.99\times10^{-17} < 0.05$, n = 23), and altitude of 4300 m (1.13 ± 0.34 *vs.* 4.38 ± 1.24, $P = 5.82\times10^{-20} < 0.05$, $n = 23$) after 6 hours of high-altitude exposure (*table 1*).

Comparison of the cytokine profiles between AMS-susceptible and AMS-resistant individuals at low altitude

To identify AMS-associated biomarkers in non-stimulated conditions, the plasma cytokine profiles from AMS-susceptible individuals and AMS-resistant individuals at low altitude were tested. In contrast to AMS-resistant individuals, there were 58 cytokines upregulated and 17 cytokines downregulated in AMS-resistant individuals (fold change ≥ 2, $P < 0.05$) (*table 2*).

Quantification of cytokines in AMS-susceptible and AMS-resistant individuals

To verify our results from the cytokine profiles, we used the quantifiable human custom cytokine antibody array to determine differentially expressed cytokines between AMS-susceptible and AMS-resistant individuals. We found that insulin-like growth factor binding protein 6 (IGFB6) (25665.38 ± 25691.29 pg/mL in AMS-susceptible individuals *vs.* 37318.99 ± 23493.11 pg/mL in AMS-resistant individuals, $P = 0.04$) was down-regulated in AMS-susceptible individuals compared with AMS-resistant individuals. In contrast to AMS-resistant individuals, serum amyloid A1(SAA1) (4069.69 ± 2502.93 pg/mL in AMS-susceptible individuals *vs.* 2994.98 ± 2295.91 pg/mL, in AMS-resistant individuals, $P = 0.05$), dickkopf WNT signaling pathway inhibitor 4 (Dkk4) (2090.00 ± 2094.89 pg/mL in AMS-susceptible individuals *vs.* 1049.88 ± 1690.93 pg/mL in AMS-resistant individuals, $P = 0.07$), and interleukin 17 receptor A (IL-17RA) (11.52 ± 8.33 pg/mL in AMS-susceptible individuals *vs.* 8.67 ± 6.22 pg/mL in AMS-resistant individuals, $P = 0.08$) were upregulated in AMS-susceptible individuals (*figure 1*).

Table 1
LLQ scores of subjects after exposure to high altitude.

Variable	AMS resistant		AMS susceptible		*P* Value
	Mean	SD	Mean	SD	
Part 1 of study ($n = 7$)					
6 hours	1.00	0.82	3.00	1.00	0.0015
9 hours	0.43	0.53	6.29	1.80	2.72×10^{-6}
12 hours	1.14	1.22	4.29	2.06	0.0046
Part 2 of study ($n = 23$)					
Altitude of 3100 m	0.39	0.50	2.79	1.25	5.13×10^{-11}
Altitude of 3500 m	0.13	0.34	3.21	1.06	3.99×10^{-17}
Altitude of 4300 m	0.13	0.34	4.38	1.24	5.82×10^{-20}

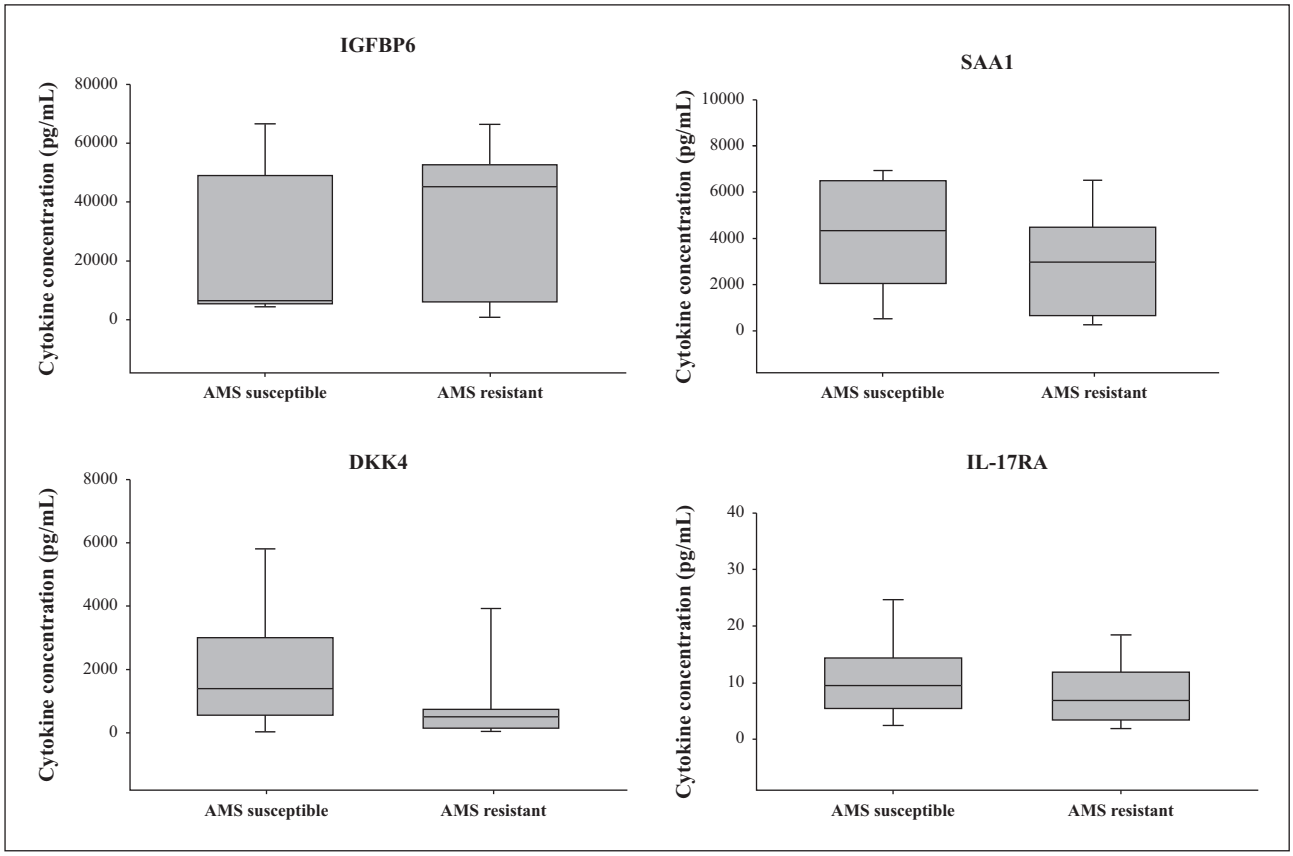

Figure 1
Plasma levels of cytokines IGFBP6, SAA1, Dkk4, and IL-17RA in AMS-susceptible individuals *vs.* AMS-resistant individuals in a low-altitude environment.

DISCUSSION

Identifying the cytokines involved in AMS is likely to shed light on the mechanisms of AMS. One recent study of AMS in a hypoxic chamber showed that AMS is closely related to inflammatory processes [10]. Our present plasma cytokine array study of the Han Chinese volunteers provides new data on a cytokine expression pattern that correlates with AMS and provides clues to predict AMS-susceptibility at low altitude. In the present study, we examined plasma cytokines of AMS-susceptible and AMS-resistant individuals at low altitude. We found that certain plasma cytokines of AMS-susceptible individuals and AMS-resistant individuals were differentially expressed at low altitude. These cytokines may therefore help to predict the incidence of AMS in conditions of low altitude. Subsequently, by further checking and quantifying these cytokines, we confirmed the differential levels of IGFBP6, SAA1, Dkk4, and IL-17RA that may serve as biomarkers to predict AMS in conditions of low altitude.

Pathophysiology of AMS

Although many theories explaining the development of AMS have been proposed during the past decades, the basic pathogenic mechanism of AMS is still fairly unclear. The blood brain barrier (BBB) theory, one hypothesis suggests that hypoxia-induced hypoxemia will initiate an inflammatory response with the release of inflammatory mediators that contribute to an increase of the capillary pressure by over-perfusion and vasodilatation, and elevate the permeability of the BBB by disrupting it. This increases the potential for capillary leak and cerebral edema, which in turn causes the traction of the meninges and blood vessels, and high-altitude headache [16-18]. According to present evidence, inflammation response may play important roles in the development of AMS. Our study suggests that AMS-resistant individuals might have more ability to respond to hypoxia *via* inflammatory processes than AMS-susceptible individuals.

Predicting AMS

Several studies have suggested that the cold presser test (CPT), heart rate variability (HRV), and lung functions may be employed as references to help predict AMS-susceptibility without reliance on simulated or actual exposure to high altitudes [19]. However, studies aimed at predicting biomarkers of AMS under such conditions are scarce.

In the present study, we made an attempt to characterise AMS-susceptible individuals based on plasma cytokine levels under low-altitude conditions. Classifying individuals as AMS-susceptible or AMS-resistant is important for those who ascend to high altitude in order to avoid toxicity effects of drugs and provide special protection. In the present study, we found that 75 cytokines were differentially expressed between the AMS-susceptible group and AMS-resistant group. Most of these cytokines are involved in inflammation. Cytokines that clearly correlated with AMS were further selected based on the quantifiable cytokines that could be predictive of AMS based on the first part of the study involving the quantifiable human custom cytokine antibody array. The levels of SAA1, Dkk4,

Table 2
The different expression levels of cytokines in plasma between AMS-susceptible and AMS-resistant individuals at low altitude.

No.	Cytokines	AMS susceptible-LA/AMS resistant-LA
1	AgRP	0.03
2	CCL16	0.04
3	FGF-8	0.05
4	IL-12 R beta 1	0.14
5	B7-1 /CD80	0.15
6	TNFRSF11A	0.23
7	Thrombopoietin (TPO)	0.31
8	CLC	0.31
9	Flt-3 Ligand	0.35
10	TLR3	0.38
11	Eotaxin-3	0.40
12	RELM beta	0.41
13	LFA-1 alpha	0.42
14	Follistatin	0.43
15	XEDAR	0.45
16	MMP-1	0.47
17	Dkk-4	0.48
18	Activin B	2.41
19	CCN4	2.77
20	CD106	20.25
21	CD170	2.21
22	Chem R23	2.99
23	EGF	9.50
24	FGF-12	2.17
25	FGF-BP	6.00
26	IFN-alpha / beta R1	2.36
27	IGFBP-6	2.37
28	IL-3 R alpha	2.12
29	IL-17R	2.10
30	MCP-3	2.06
31	MICA	2.05
32	MMP-12	2.57
33	MMP-19	2.11
34	MMP-3	28.51
35	NeuroD1	2.00
36	NRG1 Isoform GGF2	2.45
37	Orexin B	7.83
38	PDGF R alpha	2.17
39	PDGF R beta	2.49
40	PDGF-C	2.25
41	PDGF-D	2.63
42	Prolactin	2.21
43	RAGE	3.68
44	SAA	2.58
45	sFRP-1	2.10
46	Siglec-9	2.43
47	Smad 7	3.09
48	TGF-alpha	2.40
49	TGF-beta 3	8.70
50	TGF-beta RII	14.25
51	TGF-beta RIIb	2.52
52	Thrombospondin-4	27.01
53	Thymopoietin	2.45
54	Tie-1	2.29
55	TIMP-1	4.80
56	TIMP-3	2.40
57	TLR1	3.20
58	TNFSF4	2.46
59	TNFRSF7	2.20
60	TNFSF10	31.51
61	TNFRSF10B	2.86
62	TNFRSF10D	20.25
63	TNFSF12	2.48
64	TNFSF14	3.18
65	TNFRSF25	2.44
66	TRADD	2.21
67	TREM-1	2.31
68	TSG-6	46.51
69	TSLP R	2.91
70	Ubiquitin+1	2.76
71	VE-Cadherin	2.00
72	VEGF-B	3.00
73	VEGF-C	2.00
74	VEGF-D	2.52
75	WIF-1	2.55

and IL-17RA were higher, and IGFBP6 lower, in AMS-susceptible individuals than in AMS-resistant individuals. Dkk4 is an inhibitor of the WNT signalling pathway, however, its function in hypoxia or AMS is still unclear. The functions of IGFBP6 and SAA1 in AMS also remain elusive. Although the exact mechanisms of AMS are unclear, these different cytokines may serve as biomarkers to identify AMS-susceptible individuals planning to ascend to high altitudes. However, in-depth confirmation of this is required.

IL-17RA

The inflammatory response process may be implicated in the development of AMS as discussed above. The interaction between IL-17A and IL-17RA can activate the signal pathways of IL-17, which induce the ACT1/TRAF6/TRAF3-dependent pathway, which

subsequently results in the activation of the nuclear factor kappa beta (NF-KB) pathway [20], a critical signal pathway in inflammation. A significant cross-talk between NF-KB and hypoxia-induced factors (HIFs) exists, with NF-KB causing nonhypoxic upregulation of HIFs and HIFs stimulating NF-KB [21]. One recent study showed that Tibetans living at sea level have a hyporesponsive HIF signalling pathway and suppressed physiological responses to hypoxia [22]. These results suggested that the HIF signalling pathway may be involved in the development of AMS. Our present study shows that plasma levels of IL-17R are higher in AMS-susceptible individuals than in AMS-resistant individuals. This point suggests that signal pathways of IL-17, NF-KB, and HIFs are hyperresponsive in AMS-susceptible individuals, which may be responsible for AMS-susceptibility. IL-17RA may therefore be implicated as a predictor of AMS-susceptibility at low altitude.

As discussed above, impairment of the integrity of the blood-brain barrier (BBB) may play a crucial role in the development of AMS. IL-17A can interact with IL-17RA and then play important roles in various physiological processes. One study showed that BSA permeability of human brain endothelial cells increased after being incubated with IL-17A and suggested that IL-17A was implicated in the disruption of the BBB [23]. Another study showed that IL-17A can induce BBB breakdown through induced reactive oxygen species (ROS) production, which depended on NADPH oxidase and xanthine oxidase. ROS production is responsible for downregulation of tight junction molecules and activation of the endothelial contractile machinery, resulting in barrier permeabilization [24]. In our present study, we found that plasma levels of IL-17RA in AMS-susceptible individuals were higher than in AMS-resistant individuals in low-altitude conditions. This suggests that AMS-susceptible individuals may experience BBB breakdown more easily than AMS-resistant individuals. Although more in-depth studies on IL-17RA are needed, our results suggest that IL-17RA may be a biomarker to predict AMS in conditions of low altitude.

Study limitations

Here, we should address some limitations of our present study. First, the sample was from the peripheral blood. As mentioned in a recent study, plasma may not accurately represent the cerebral environment, however, peripherally circulating proteins affect cerebral endothelial permeability [25], suggesting that cytokines in plasma could affect AMS. In the present study, the investigation of AMS biomarkers was limited to peripheral blood samples. Second, we analyzed sample pooling using cytokine array analysis in the first part of the study. A comprehensive evaluation of the effect of sample pooling for proteomic analysis demonstrated that, for the majority of proteins, data obtained from pooled samples accurately represented the mean protein levels of individual samples [26]. This strategy is advantageous when the research focus is on elucidating common features of a given population or disease group [27], as was the case in our study. However, the results based on the sample pooling analysis methods require further in-depth studies. Finally, although our work has provided some important clues, further confirmation of the cytokine array analysis was not performed on animals or cell models. The primary reason for this is based on the subjective symptoms of AMS. Coupled with the elusive mechanisms of AMS, especially regarding high-altitude headache, we cannot model AMS in animals. To further test the cytokine array analysis results in humans, a large sample is required, however, this may raise ethical issues.

CONCLUSIONS

In our present study, we identified the levels of four different cytokines, IGFBP6, Dkk4, SAA1, and IL-17RA, which were significantly differentially expressed between AMS-resistant individuals and AMS-susceptible individuals at low altitude. Although further in-depth research is needed, these four cytokines could prove to be useful in predicting AMS-susceptibility in low-altitude environments.

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REFERENCES

1. West JB, American College of P. JB, American Physiological S. JB. The physiologic basis of high-altitude diseases. *Ann Intern Med* 2004; 141: 789-800.
2. Bartsch P, Swenson ER. Clinical practice: acute high-altitude illnesses. *N Engl J Med* 2013; 368: 2294-302.
3. Kayser B, Dumont L, Lysakowski C, Combescure C, Haller G, Tramer MR. Reappraisal of acetazolamide for the prevention of acute mountain sickness: a systematic review and meta-analysis. *High Alt Med Biol* 2012; 13: 82-92.
4. Tang E, Chen Y, Luo Y. Dexamethasone for the prevention of acute mountain sickness: systematic review and meta-analysis. *Int J Cardiol* 2014; 173: 133-8.
5. Dumont L, Mardirosoff C, Tramer MR. Efficacy and harm of pharmacological prevention of acute mountain sickness: quantitative systematic review. *BMJ* 2000; 321: 267-72.
6. Ellsworth AJ, Larson EB, Strickland D. A randomized trial of dexamethasone and acetazolamide for acute mountain sickness prophylaxis. *Am J Med* 1987; 83: 1024-30.
7. Subedi BH, Pokharel J, Goodman TL, *et al*. Complications of steroid use on Mt. Everest. *Wilderness Environ Med* 2010; 21: 345-8.
8. Julian CG, Subudhi AW, Hill RC, *et al*. Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. *J Appl Physiol* 2014; 116: 937-44 (1985).
9. MacInnis MJ, Koehle MS, Rupert JL. Evidence for a genetic basis for altitude illness: 2010 update. *High Alt Med Biol* 2010; 11: 349-68.
10. Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, Roach RC. Acute mountain sickness, inflammation, and permeability: new insights from a blood biomarker study. *J Appl Physiol* 2011; 111: 392-9 (1985).
11. Chiu TF, Chen LL, Su DH, *et al*. Rhodiola crenulata extract for prevention of acute mountain sickness: a randomized, double-blind, placebo-controlled, crossover trial. *BMC Complement Altern Med* 2013; 13: 298.

12. Roach RC, Maes D, Sandoval D, *et al*. Exercise exacerbates acute mountain sickness at simulated high altitude. *J Appl Physiol* 2000; 88: 581-5 (1985).

13. Rink C, Khanna S. Significance of brain tissue oxygenation and the arachidonic acid cascade in stroke. *Antioxid Redox Signal* 2011; 14: 1889-903.

14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-54.

15. Zor T, Selinger Z. Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. *Anal Biochem* 1996; 236: 302-8.

16. Hackett PH, Roach RC. High-altitude illness. *N Engl J Med* 2001; 345: 107-14.

17. Hartmann G, Tschop M, Fischer R, *et al*. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine* 2000; 12: 246-52.

18. Van Osta A, Moraine JJ, Melot C, Mairbaurl H, Maggiorini M, Naeije R. Effects of high altitude exposure on cerebral hemodynamics in normal subjects. *Stroke* 2005; 36: 557-60.

19. Song H, Ke T, Luo WJ, Chen JY. Non-high altitude methods for rapid screening of susceptibility to acute mountain sickness. *BMC Public Health* 2013; 13: 902.

20. Xu S, Cao X. Interleukin-17 and its expanding biological functions. *Cell Mol Immunol* 2010; 7: 164-74.

21. Bruning U, Fitzpatrick SF, Frank T, Birtwistle M, Taylor CT, Cheong A. NFkappaB and HIF display synergistic behaviour during hypoxic inflammation. *Cell Mol Life Sci* 2012; 69: 1319-29.

22. Petousi N, Croft QP, Cavalleri GL, *et al*. Tibetans living at sea level have a hyporesponsive hypoxia-inducible factor system and blunted physiological responses to hypoxia. *J Appl Physiol* 2014; 116: 893-904 (1985).

23. Kebir H, Kreymborg K, Ifergan I, *et al*. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 2007; 13: 1173-5.

24. Huppert J, Closhen D, Croxford A, *et al*. Cellular mechanisms of IL-17-induced blood-brain barrier disruption. *FASEB J* 2010; 24: 1023-34.

25. Huber JD, Egleton RD, Davis TP. Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends Neurosci* 2001; 24: 719-25.

26. Diz AP, Truebano M, Skibinski DO. The consequences of sample pooling in proteomics: an empirical study. *Electrophoresis* 2009; 30: 2967-75.

27. Karp NA, Lilley KS. Investigating sample pooling strategies for DIGE experiments to address biological variability. *Proteomics* 2009; 9: 388-97.