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CROSSTALK BETWEEN HUMAN BODY CONSTITUTIONAL TYPES IN MONGOLIAN TRADITIONAL MEDICINE AND IMMUNE RESPONSE TYPES

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ПЕРЕКРЕСТНЫЕ СВЯЗИ МЕЖДУ КОНСТИТУЦИОНАЛЬНЫМИ ТИПАМИ ЧЕЛОВЕЧЕСКОГО ТЕЛА В МОНГОЛЬСКОЙ НАРОДНОЙ МЕДИЦИНЕ И ТИПАМИ ИММУННОГО ОТВЕТА

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Abstract. Human constitutional typology system in Traditional Mongolian Medicine (TMM) explains not only the differences in physical characteristics, but somatic, mental, spiritual and behavioral peculiarities of individuals and as well as environmental factors influencing on their existence. In TMM the typology system prescribes several human constitutional types in accordance with domination of abstraction substances (or humors, or notions, or elements) Khii, Shar and Badgan and it may be specified into 3 single, 3 mixed or mixed and one collected human constitutional types (Figure 1) and herewith each person have a own proportion of these substances determined at the birth, tough the proportion may have to changed during the lifetime under influence of both internal and external factors, including aging, harmful and pathogenic factors. This review is aimed to analyze and summarize results of research works developed at the Mongolian National University of Medical Sciences (MNUMS) and which were focused on relationship between traditional and modern medicines.

There were 3 independent research and development projects was focused on relationship between types of immune response and typology systems in TMM at the MNUMS during 2010–2019. In cytokine study project done in 93 blood donors were found low titer of pro-inflammatory cytokines, respectively low activation potential of T helper 1 (Th1) mediated inflammation has related with Badgan humor domination, but relation of Shar humor with anti-inflammatory cytokines, therefore with Th^2 mediated response was remained open. In chronic hepatitis B study were enrolled 101 patients with certain phases of this pathology and established 1) immune active phase or condition of CHB may be related with Khii humor; 2) reactivation phase/condition of CHB may be related with Shar humor; and 3) inactive phase/condition — with Badgan humor. Study of peripheral blood white cells count in 287 blood donors demonstrated possible relationship between CD4+ T cell count and Badgan humor, and iNKT cell count and Khii humor. Study of serum immunoglobulin and compliment titer was performed in 242 blood donors and demonstrated significantly higher C3 titer in subjects with Badgan dominated humors. Also were shown direct intermediate correlation between titer of serum IgG and IgM and Shar humor expression score, and between titer of serum C3 and IgM Badgan humor expression score.

On the basis of these findings were suggested: 1) Shar humor expression has an association with Th^2 mediated adaptive immune response; 2) Badgan humor expression has an association with Th1 mediated response; 3) and Khii humor has an intermediate position regulating Shar and Badgan humor expression and has an association with NKT cells, which likely to regulate Th1 and Th^2 mediated response activity. To ensure or decline above mentioned hypothesis there is required to continue and expand the research in this field.

Key words: Mongolian traditional medicine, human body constitution, immune response type

Аннотация. Система конституциональной типологии человека в традиционной монгольской медицине (ТММ) объясняет не только различия в физических характеристиках, но и соматические, психические, духовные и поведенческие особенности индивидов, а также факторы окружающей среды, влияющие на их существование. В ТММ система типологии предписывает несколько конституциональных типов человека в соответствии с доминированием абстрагирующих веществ (или юморов, или понятий, или элементов) Khii, Shar и Badgan, и она может быть указана на 3 отдельных, 3 смешанных или смешанных и один собранный человеческий конституциональный. типы (рис. 1), и при этом у каждого человека есть своя пропорция этих веществ, определенная при рождении, хотя пропорция может измениться в течение жизни под воздействием как внутренних, так и внешних факторов, включая старение, вредные и патогенные факторы. Целью данного обзора является анализ и обобщение результатов научных исследований, разработанных в Монгольском национальном университете медицинских наук (MNUMS) и посвященных взаимосвязи между традиционными и современными лекарственными средствами.

Было 3 независимых исследовательских и опытно-конструкторских проекта, посвященных взаимосвязи между типами иммунного ответа и типологическими системами в ТММ в MNUMS в течение 2010–2019 гг. В проекте исследования цитокинов, выполненном у 93 доноров крови, был обнаружен низкий титр провоспалительных цитокинов, соответственно низкий потенциал активации воспаления, опосредованного Т-хелпером 1 (Th1), связан с доминированием юмора Бадгана, но связь юмора Шар с противовоспалительными цитокинами, поэтому с Th2 опосредованный ответ оставался открытым. В исследование хронического гепатита В были включены 101 пациент с определенными фазами этой патологии и установлено 1) иммунно-активная фаза или состояние ХГБ могут быть связаны с юмором Khii; 2) фаза реактивации / состояние ХГВ могут быть связаны с шарм юмором; и 3) неактивная фаза / состояние — с юмором Badgan. Исследование количества лейкоцитов периферической крови у 287 доноров крови продемонстрировало возможную связь между количеством CD4 + Т-кле-

ток и юмором Бадгана, а также количеством клеток iNKT и юмором Khii. Исследование сывороточного иммуноглобулина и титра комплемента было выполнено у 242 доноров крови и продемонстрировало значительно более высокий титр С3 у субъектов с юмором, в котором доминировали бадганы. Также была показана прямая промежуточная корреляция между титром сывороточных IgG и IgM и оценкой экспрессии юмора по Шар-шу, а также между титром сывороточной С3 и балльной оценкой юмора по Бадгану IgM.

На основании этих результатов были предложены: 1) экспрессия шар-юмора связана с Th2-опосредованным адаптивным иммунным ответом; 2) Бадганская экспрессия юмора связана с Th1-опосредованным ответом; 3) и юмор Khii имеет промежуточное положение, регулирующее экспрессию юмора Shar и Badgan, и имеет связь с клетками NKT, которые, вероятно, регулируют активность ответа, опосредованную Th1 и Th2. Чтобы обеспечить или опровергнуть вышеупомянутую гипотезу, необходимо продолжить и расширить исследования в этой области.

Ключевые слова: монгольская народная медицина, конституция тела человека, тип иммунного ответа.

Background.

Human typology in Traditional Mongolian Medicine. Human constitutional typology system (in cyrillic Mongolian — өвөрчлөл [uburchlul], in classical Mongolian - ᠥᠪᠥᠷᠴᠯᠥᠯ) in Traditional Mongolian Medicine (TMM) has been based in constitutional concepts of ancient Indian and traditional Tibetan medicine and have been adapted into the culture of nomadic Mongolian people living in Central Asian continental plateau, and developed last several centuries with regard to lifestyle of this people [2]. This typology system explains not only the differences in physical characteristics, but somatic, mental, spiritual and behavioral peculiarities of individuals and as well as environmental factors influencing on their existence [2–4].

In TMM the typology system prescribes several human constitutional types in accordance with domination of abstraction substances (or humors, or notions, or elements) in human body. These substances are "Khii" (ཁྱུ, Tibetan-*rlung [loong]* or wind, or vital energy, or air), "Shar" (ཞར་བ, Tibetan-*mkrispa [kris-pa], or Tripa* or *mucus, or fire*) and "Badgan" (བད་ཀན, Tibetan-*badken or pekan,* or *phlegm,* or *bile, or earth/water*) and it may be specified into 3 single, 3 mixed or mixed and one collected human constitutional types (Figure 1) and herewith each person have a own proportion of these substances determined at the birth, tough the proportion may have to changed during the lifetime under influence of both internal and external factors, including aging, harmful and pathogenic factors [3, 5–7].

Definition of human constitutional types. Human constitutional typology is widely used for differential approaches of diagnostics and treatment of diseases in TMM. It became possible after adaptation and development of test system for constitutional typing in Research, Technology and Manufacturer Corporation of Traditional Medicine of Mongolia (2003) [3]. Primary version of this scoring test system was developed by Sachs R (1995) [6].

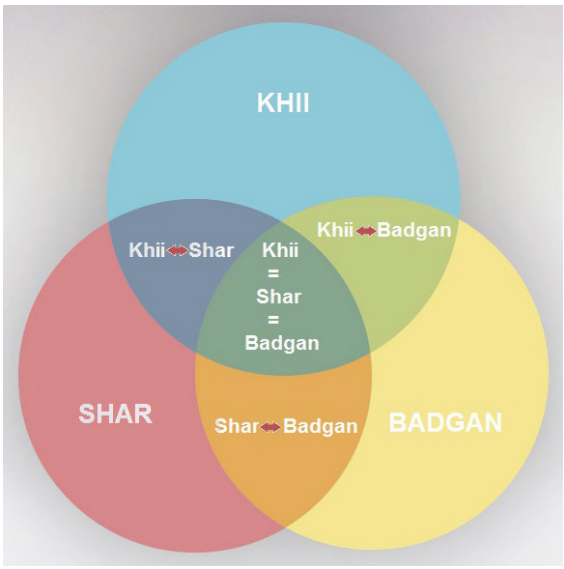

Figure 1. Human body constitution concept in Traditional Mongolian Medicine

The system includes 100 tests for collection of data using findings from anamnesis, observation and physical examination and all these tests were grouped in four sections such as body characteristics (25 tests), general properties (15 tests), individual's imagination (41 tests) and special properties (19 tests). Test results expressed with scores filled in three columns corresponding to Khii, Shar and Badgan humors. According to this test system constitution of each donor was defined by calculation of scores using a special formula. There may be specified 3 single, 3 (6) mixed and 3 collected types of human constitution and 3 dominantly expressed humor in each person. Dominating humors were defined as follows: Khii dominated humor includes single Khii, Khii-Badgan and Khii-Shar mixed types; Shar dominated humor includes single Shar, Shar-Badgan and Shar-Khii mixed types; and Badgan dominated humor includes single Badgan, Badgan-Shar and Badgan-Khii mixed types.

Human constitutional typology in Oriental medicine and its interpretation. A number of reports have been dedicated to the possible relationship between basic concepts of Oriental Traditional Medicines and modern medicine. In particular, relationship of modern medicine with Sasang typology in Korean medicine [8–10], Yin-Yang typology in Chinese medicine [9, 11], typology in Ayurveda medicine [12] and TMM [7, 13, 14] have been discussed. Mongolian author Ambaga M (2017) [15] suggested that behind all the concepts around living rLung, Mkhris, Badgan in traditional Mongolian medicine are due to regulations in the membrane — redox potentials three — state line system dependent — full 9 stepped cycle of proton conductance inside human body. We did not find results of other studies describing relationship between types of adaptive immune response and types of human body typology used in other traditional Oriental medicine. However some studies reported the relationship between human body constitution types and certain pathologies. Sohn KW et al. (2012) in their meta-analysis reported the significant difference among Sasang types of typology system used in Korean traditional medicine in distribution of genotypes or haplotypes related with development of coronary and metabolic diseases, HLA phenotypes and drug resistance [8]. Wan Q et al. (2010) observed no significant difference in mean titer of IL-1β and IL-2 in subjects with yin-deficiency constitution and gentle constitution according Chinese traditional medicine, although yin-deficiency subjects demonstrated increased level of cortisol, adrenocorticotropic hormone and cyclic guanosine monophosphate compared to subjects with gentle constitution [11].

The immune response. The immune system in mammalians is the organ system responsible for antigenic environment in the body and the immune response is the principal mechanism to establish optimal antigenic homeostasis. The types, forms and efficacy of the specific immune response triggered by foreign antigen exposure are correlated, on one hand, from natural properties of the exposed antigen, and its quantity and route of exposure and on other hand by the individual peculiarities of mammalian host [16, 17]. The end result of the immune response to microbial antigens in individuals can determine, for example, their inheritedand/or acquired susceptibility or resistance to parasitic, viral and bacterial infections [18–22].

There are two main subsets of T lymphocytes, distinguished by the presence of cell surface molecules known as CD4 and CD8. T lymphocytes expressing CD4 are also known as helper T cells, and these are regarded as being the most prolific cytokine producers. This subset can be further subdivided into Th1 and Th2, and the cytokines they produce are known as Th1-type cytokines and Th2-type cytokines. Th1-type cytokines tend to produce the proinflammatory responses responsible for killing intracellular parasites and for perpetuating autoimmune responses. Interferon gamma is the main Th1 cytokine. Excessive proinflammatory responses can lead to uncontrolled tissue damage, so there needs to be a mechanism to counteract this [23]. The Th2-type cytokines include interleukins 4, 5, and 13, which are associated with the promotion of IgE and eosinophilic responses in atopy, and also interleukin-10, which has more of an anti-inflammatory response. In excess, Th2 responses will counteract the Th1 mediated microbicidal action. The optimal scenario would therefore seem to be that humans should produce a well

balanced Th1 and Th2 response, suited to the immune challenge. Many researchers regard allergy as a Th2 weighted imbalance, and recently immunologists have been investigating ways to redirect allergic Th2 responses in favour of Th1 responses to try to reduce the incidence of atopy. Some groups have been looking at using high dose exposure to allergen to drive up the Th1 response in established disease [24], and other groups have been studying the use of mycobacterial vaccines in an attempt to drive a stronger Th1 response in early life [25].

This review is aimed to analyze and summarize results of research works developed at the Mongolian National University of Medical Sciences (MNUMS) and which were focused on relationship between traditional and modern medicines.

What we did find and how to understand it?

There were 3 independent research and development projects was focused on relationship between types of immune response and typology systems in TMM at the MNUMS during 2010–2019.

Cytokine study

First 3 year grant (2010–2013) was financed by Science and Technology Foundation, the government agency for support the research and innovation in the country. In this cross-sectional study enrolled 93 blood donors and it was aimed to establish possible relationship between human typology and immune response types. Study was designed to establish constitutional types and titer (pg/mL) of common pro-inflammatory (IL-2, IL-6 and IFNγ) and anti-inflammatory (TGFβ, and IL10) cytokines in both peripheral blood plasma and peripheral blood lymphocyte overnight culture supernatant [26]. Main findings of the study were as follows:

Pro-inflammatory cytokines. Were found elevated mean IL-2 titer in Shar-Badgan type subjects comparing with single Badgan type, decreased plasma IL-2 mean titer in Badgan humor dominated subjects comparing with Shar (p=0.027) and Khii humor dominated subjects (p=0.001), and lower supernatant mean IL-2 titer in Badgan humor dominated subjects comparing with Shar humor dominated subjects. Were observed lower plasma IL-6 mean titer in subjects with single Badgan type compared with Khii-Shar mixed type subjects and lower supernatant IL-6 level in Badgan humor dominated donors compared with Khii humor dominated donors (p=0.027). Single Shar type subjects had a decreased mean titer of supernatant IFNγ compared with Shar-Badgan mixed type subjects. In our study we observed decreased level of plasma IL-2 (10.5±1.79 vs 13.5±5.00 pg/mL; p=0.008) IL-6 (6.5±0.39 vs 8.9±3.66 pg/mL; p=0.021) and supernatant IFNγ (79.3±10.25 vs 104.1±29.8 pg/mL; p=0.045) in subjects aged 45 year and elder (n=16) compared to younger than 45 year subjects (n=68). In other side Khii expression score has shown indirect correlation with age of donors (r= –0.118, p=0.046) [unpublished data].

Interleukin-2 plays a pivotal role in the immune response. It is a growth factor that promotes NK cell activity and the differentiation of naïve T cells into Th1 and Th2 cells [27]. Conversely, IL-2, acting *via* STAT5 pathway negatively regulates interleukin 17 (IL-17) production [28]. Most studies show that lymphocytes in elderly people produce significantly less IL-2, compared to young people [29]. Intracellular cytokine studies have shown variable results for IL-2, whereas mitogen-induced stimulation of mononuclear cells from elderly subjects showed significant decreases in IL-2 and IFNγ production [30, 31].

Anti-inflammatory cytokines. IL-10 levels showed higher a mean titer in single Shar type subjects compared to Shar-Badgan mixed type subjects.

Here we can see that low titer of pro-inflammatory cytokines, respectively low activation potential of T helper 1 (Th1) mediated inflammation has related with Badgan humor domination, but relation of Shar humor with anti-inflammatory cytokines, therefore with Th2 mediated response was remained open.

Chronic hepatitis B study.

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV) presenting a major global health problem and it can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer [32]. Mongolia is the country with high rate of hepatitis B disease burden with 10.6% of positive Hepatitis B surface Antigen (HBsAg) and 78.1 age-

standardized incidence rate of liver cancer per 100, 000 population [33].

In this cross-sectional study were enrolled 101 (53 males and 48 females) patients aged 21–81 year with established state of chronic hepatitis B (CHB) [34]. Staging of chronic HBV infection was performed according to criteria specified in "Guideline for Detection, Diagnosis and Treatment of hepatitis B (HBV) and hepatitis D (HDV) virus infection. 2016" approved by Ministry of Health, Mongolia [35]. The guideline was developed in compliance with WHO Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection (2015) [36]. There were selected 101 patients in certain three phases of chronic HBV: in immune active, immune inactive and immune reactivation phases and without mix (hepatitis A and/or hepatitis C virus) and/or co-infection (hepatitis D virus).

There was demonstrated significantly increased distribution of inactive CHB cases among single Badgan type patients and reactive HBV infection among single Shar and Shar-Khii type patients (χ^2=69.5; p=0.001). Furthermore, distribution of constitutional types by dominantly expressed humors among patients with CHB has revealed with significant difference, namely, significantly high portion of patients with active phases of chronic HBV infection was demonstrated Khii dominated humors, while more patients with reactivation phases demonstrated Shar dominated humors and patients with inactive phases — Badgan dominated humors (χ^2=55.4; p=0.001). Mean value of serum ALT and AST in Badgan dominated humor patients was lower compared to that in Khii and Shar humor dominated patients. More patients (16 of 23) with Khii dominated humors demonstrated positive HBeAg, while more patients (21 of 30) with Badgan dominated humors had a seroconversion for HBeAg (χ^2=9.4; p=0.009) and Shar dominated patients has a similar portion of HBeAg positive or negative patients (28 and 20 respectively).

Tibetan classical manuscript *"Gyud-shi" describes physical cause of human typology as follows* «… the physical or material causes of the three humors are: the subtle wind element … becomes the cause of the Wind (Khii) humor and the nervous system; the sperm, which is the cause of the Phlegm (Badgan) humor and of the lymphatic and endocrine systems; the menstrual blood (ovum), which is the cause of the Bile (Shar) humor, the blood and blood circulation and of the metabolic systems. The three humors … develop the body, and govern the body/mind and its functions. They rule physiology, anatomy and morphology, regulate the functioning of the body, its organs, the brain, nerves, bones, blood circulation, lymphatic systems, digestion etc. Balanced humors give positive health and harmony to the body/mind, and provide a good base for the development of the body/mind, the immune system and protection. On the contrary, the loss of balance among the humors causes energy disharmony and physical and mental disequilibrium which may appear at any time and become the cause of diseases …" [37]. If we will look trough prism of balanced humors we will summarize key findings of the current study as follows: distribution of human constitutional types used for differential approach of diagnostics and treatment of diseases in traditional Mongolian medicine is significantly different among patients with different phases of chronic hepatitis B. Here we can suggest next statements: 1) immune active phase or condition of CHB may be related with Khii humor; 2) reactivation phase/condition of CHB may be related with Shar humor; and 3) inactive phase/condition — with Badgan humor. Balmasova IP, et al. (2014) [38] reviewed literature reports focused on immunopathogenesis of CHB and described mechanisms of innate and adaptive immunity, which involved in different stages of chronic HBV infection. According to this report active phase of CHB characterized by signs of active liver inflammation in liver histology with elevated infiltration of intrahepatic CD4+ T helper 1 (Th1) and CD8+ cytotoxic (CTLs) lymphocytes, high content of Th17 cells in the liver and in the blood, and decreased in the liver and increased in the blood count of regulatory T cells (Treg). In contrast, inactive phase of CHB characterized by signs of mild inflammation or inactive cirrhosis in liver histology with predominance of inflammatory Th1 cells in liver and low HBV-specific immune response. But patients with reactivation phase of chronic HBV infection have demonstrated signs of normal tissue or cirrhosis and hepatocellular

carcinoma in liver histology, predominance of inflammatory Th1 cells and fallen down CD8+ CTL cells. So, findings of our study in accordance with this review let us to suppose that Khii humor domination is correlated with imbalance of immune regulation toward the intensive Th1 mediated cytotoxicity due to CTLs and Th17 mediated inflammation and down regulated Th2 response due to suppressed Treg function in the tissue. In other hand Th1 mediated inflammation is powerful tool against intracellular infection [39, 40], and it may frequently affect cells and cause tissue damage, which are typical pathological findings for immune active phase of chronic HBV infection [38]. Shar humor domination is correlated with intensive Th1 mediated inflammation due to activated local macrophages (Kupffer cells) [38]. Zhang JY, et al. (2010) [41] found increased number of Th17 cells in peripheral blood and liver tissue of patients with CHB comparing with healthy liver donors and positively correlated with degree of liver injury with Th17 cell count.

If inflammation affecting liver tissue has more intensity in patients with active phase [38, 41, 42] and the tissue transforming potential is higher in reactivation phase of chronic HBV infection [38, 42] we may suggest the Shar humor may be related with more intensive tissue regeneration/remodeling potential.

Study of peripheral blood white cells count

This 2 year project (2017–2018) [43] was financed by Foundation for Support of Science and Technology of MNUMS. Total 287 blood donors were enrolled in this cross-sectional study. Human constitution types were defined according to the testing system [3]. Blood sample collected from all donors were treated with mix of fluorescent monoclonal antibodies (Biolegend, USA) against surface antigens of white blood cells. Absolute count of leukocytes (CD45+), T (CD3+), B (CD19+), natural killer (NK; CD3-CD56+) and invariant natural killer T (NKT; CD3+, TCRVα24-Jα18) lymphocytes, CD4+ and CD8+ subset of T lymphocytes (cell/μL) were measured by magnetic activated cell sorting (MACSQuant Analyzer 10, Miltenyi Biotec) assay and CD4/CD8 ratio was calculated by division of values. Percent (%) of lymphocyte subsets calculated from total lymphocyte count.

There were observed wide variation for iNKT and CD3+CD56+ cells (0–813 and 3–584 respectively) with an abnormal distribution. Interestingly, most of subjects with extremely high count of iNKT cells were investigated in May.

Mean value of CD8+ T cell count in subjects with Khii-Badgan mixed type was higher than in other type groups, besides Badgan-Shar mixed type subjects (independent-samples t test; $p<0.01$). Subjects with Badgan humor dominated constitution demonstrated significantly higher mean value of CD4/CD8 ratio compared to Khii or Badgan dominated subjects. There were demonstrated direct correlation of CD4+ cell count and CD4/CD8 ratio with expression scores of Shar and Badgan humors. Also was shown indirect correlation of Shar humor scores with B lymphocyte percentage ($r = -0.180$; $p = 0.002$) and direct correlation with T lymphocyte ($r = 0.189$; $p = 0.001$) and CD4+ lymphocyte ($r = 0.248$; $p = 0.000$) percentage. Badgan humor expression score was directly correlated with percentage of CD4+ cells ($r = 0.204$; $p = 0.001$), but indirectly with percentage of CD8+ cells ($r = -0.176$; $p = 0.003$). To clarify relationship between constitution types and count of iNKT and CD3+CD56+ cells blood donors were grouped into 4 groups by quartile growth of these cells, and in parallel into 3 groups by expression score percentage of given humor (less than 33%, 34–66% and more than 66%). Crosstabulation of these groups discovered some associations between cell count and humor expression. For example, large portion (89 of 151, or 58.9%) of donors with weak (less than 33%) expression of Khii humor demonstrated no or low (0.1–2.0 cell/μL) count of iNKT cells ($\chi^2=16.2$; $p=0.013$). Binary classification analysis of low iNKT cell count (less 2.0 cell/μL) with weak expression (less 33%) of Khii humor likewise shown significant true positive rate. In similar way were calculated association of other cell populations with humor expression and found following correlations: 1) donors with strong expression (>66%) of Shar humors were more frequently belonged (23 from 50; $\chi^2=48.6$; $p<0.001$) to high count CD4+ T cell (>1000 cell/μL) population [32]; 2) more subjects with weak expression of Badgan humors had a low count (<500 cell/μL) of CD4+ T cell (38 from 66;

χ^2=46.8; p<0.001); and 3) more subjects with weak expression of Badgan humors had a low value (<1.0) of CD4/CD8 ratio (18 from 30; χ^2=18.6; p<0.001).

We have done some attempts for paralleling of modern representation of immune response mechanisms with human constitution concept prescribed in Tibetan manuscripts. Th1 mediated response in adaptive immunity normally provides defense against intracellular infection and related with resistance to intracellular infections but people with a high intensity of Th1 mediated response may be susceptible to infections with pyogenic bacteria and their toxins [17, 39, 40]. According to English interpretation of "*The Essentials of Gyud-shi" [37]* Badgan or Phlegm humor "*… derives from the two elements of earth and water. Earth provides the quality of heaviness, and water provides the qualities of humidity and coldness. Both produce the qualities of coldness, wetness, binding and heaviness. The cool and wet nature of the Phlegm humor opposes the heat of Bile, while its qualities of heaviness and gentleness (smoothness) balance the roughness of the Wind humor. The Phlegm humor controls the Bile heat, infection and fever disorders …*".

In contrast Th2 mediated response in adaptive immunity is responsible for humoral type of immune response and normally related with resistance to infection with extracellular bacteria and fungi, and provides defense from parasite infection [17]. *The Essentials of Gyud-shi* describes Shar or Bile humor "*… heat and protects the body from the coldness and humidity of the Khii and Badgan humors, it rules metabolism, hunger, thirst, food digestion and nutrition transformation, and it gives temperature to the body*" [37].

Expression of Khii humor was directly associated with count of iNKT cells. iNKT cells are a regulatory subset of T lymphocytes whose frequency in peripheral blood is highly variable within the human population. Lower than normal NKT frequencies are associated with increased predisposition to a number of diseases, including type 1 diabetes and some forms of cancer, raising the possibility that an increased frequency may be protective. However, there is little or no understanding of how high NKT frequencies arise or, most importantly, whether the potential exists to boost and maintain NKT levels for therapeutic advantage [44]. Correlation analysis of iNKT cell count with other immune cells of peripheral blood in this study has shown its direct correlation with count and percentage of CD3+, CD4+ T cells and CD4/CD8 ratio. *The Essentials of Gyud-shi* encloses "*… Khii or Wind humor is the breath, life force, and energy of the body/mind. It is pervasive and circulates inside and outside of the body. It manifests from the mind and the subtle wind energy and it is the source of respiration, movement, strength, and the power of the body/mind. It especially governs the main function of the mind and the wind systems. Functionally, the Wind humor is a cold but neutral energy and it is equally able to associate itself with Phlegm or Bile*" [37].

It is very complicated to draw direct correlation between pathological conditions in modern European medicine and humor disorders in Tibetan and Mongolian medicine. Because of the word humor we used in this interpretation has definitely different interpretation comparing to that used in Greek and Roman medicine [45]. According to the Gyud-shi humors sustain health, keep the body systems in order, transform the nutrients into body constituents and power, and regulate the body and mind functions [37].

Study of serum immunoglobulin and compliment

Total 242 of 287 blood donors investigated for peripheral blood cell count study were studied for serum IgA, IgG, IgM and complement C3, C4 component titer. Serum IgA, IgM, IgG and C3, C4 complement (mg/dL) were measured using turbidimetric assay (Fortress diagnostics, UK). Donors with Badgan-Shar constitution demonstrated significantly higher C3 titer compared to donors with Khii-Shar constitution (p=0.013). Subjects with Badgan dominated humors has shown significantly higher C3 titer compared to Khii dominated subjects (p=0.004). Titer of serum IgG and IgM has shown direct intermediate correlation with Shar humor expression score (p=0.024) and titer of serum C3 (p=0.027) and IgM (p=0.047) was directly correlated with Badgan humor expression score (unpublished data).

Summary and Hypothesis

Analysis of above mentioned findings and citations allow us to do following suggestions:

1) Shar humor expression has an association with Th2 mediated adaptive immune response; 2) Badgan humor expression has an association with Th1 mediated response; 3) and Khii humor has an intermediate position regulating Shar and Badgan humor expression and has an association with NKT cells, which likely to regulate Th1 and Th2 mediated response activity (Figure 2).

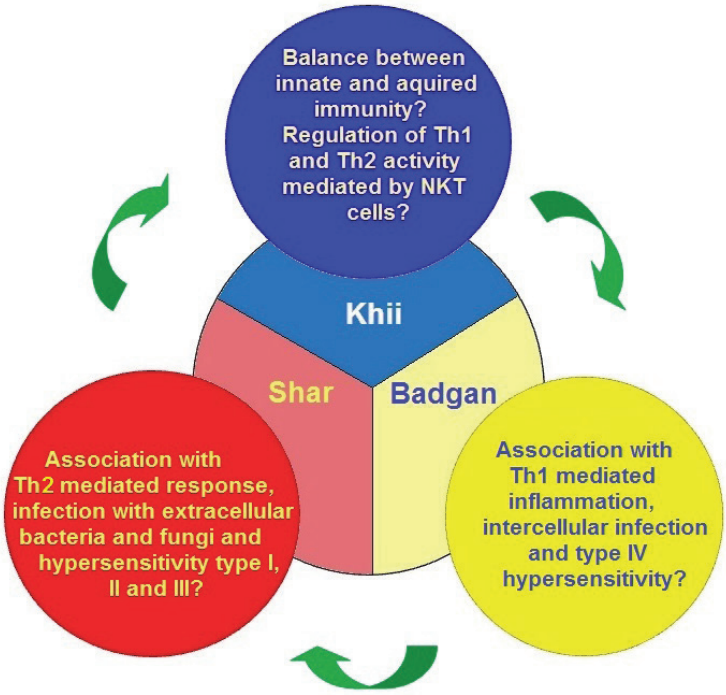

Figure 2. Possible relationship of humors determining human constitution type in TMM with types of immune response and pathological conditions caused by aberrant immune response

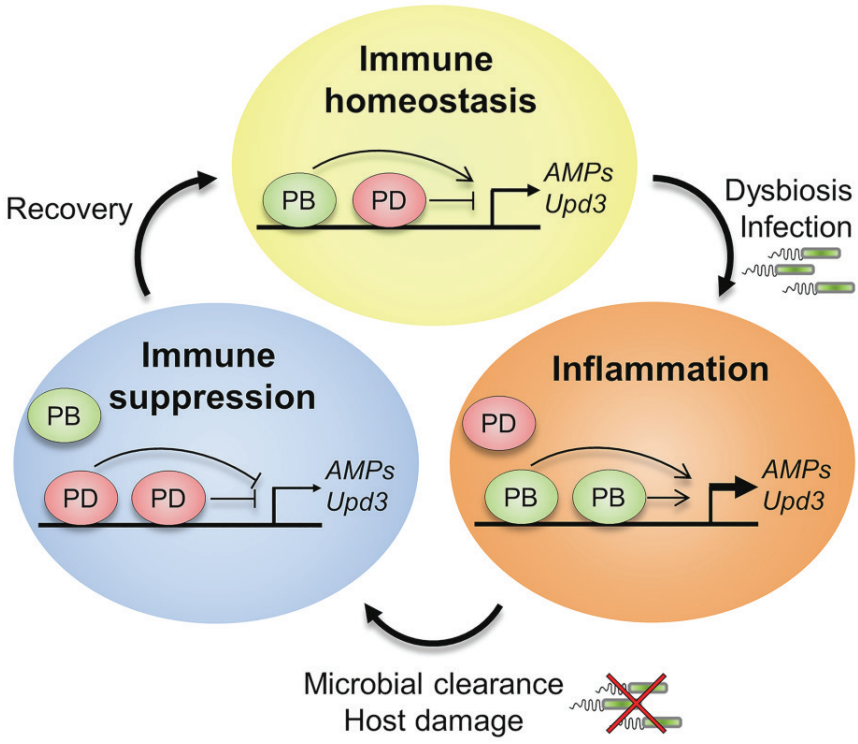

Figure 3. Model of the antagonistic actions of Nub isoforms (Lindberg BG, et al., 2018).

Very interesting observation was done after Lindberg BG, et al (2018) [46]. They studied gene isoforms participating in immune homeostatsis in *Drosophila* and found that Nub-PB and Nub-PD, which antagonistically regulate immune gene expression in Drosophila and therefore prevent aberrant immune activity (Figure 3).

During normal conditions, Nub-PD interacts with the proximal promoter region of immune-regulated genes to repress aberrant expression. Microbial dysbiosis or oral infection skews the isoform ratio towards Nub-PB, which through an unknown mechanism outcompetes Nub-PD and activates immune gene transcription. Once microbial homeostasis has been reestablished, the equilibrium between the isoforms is regained to balance gut immunity. Uncontrolled expression of Nub-PB or a lack of Nub-PD results in a hyperactivate immune response, loss of tissue homeostasis and early host death. https://doi.org/10.1371/journal.ppat.1006936.g007

According to this model the failure of immune homeostasis under exposure of both external and/or internal factors followed with two subsequent conditions — inflammation and remodeling of tissue. We see surprising similarity of this model with different humor expression in different stages of immune regulation.

Therefore do abstraction notion of humors defining the human constitutional typing in TMM present universal formula for the adaptation? So may it be that each subject have balanced, but own specific adaptation power, predicting susceptibility or resistance to certain infection or pathologic condition? If yes, do this able to point the novel personal diagnostic and therapeutic approaches?

Further considerations. We started this study supposing the human typology used in TMM may have relationship with certain types of immune responses. Our findings allow answering positively for this question, but in long run we remained with more questions than answers.

Definitely, to ensure or decline above mentioned hypothesis there is required to continue and expand the research in this field.

Firstly, we need is to continue the study in cohort design at least for 12 months follow of donors to establish or deny influence of environmental factors such as climate and infection. It would be excellent if additional functional markers to assess activation, memory and exhaust state and cytokine production of NKT cells and T cell subsets will be investigated.

Secondly, an investigation of human constitution types and its variation in sufficient number of patients with certain pathology believed to clarify many details of relationship between concepts of modern and traditional medicines. The selected pathology model, preferably inflammatory disease, should be characterized with well-described immune disorder mechanisms and with wide specter of biomarkers for control of disease course.

Thirdly, we need to improve testing system for definition of human constitutional types. Some of test items seems likely to be quite abstractive and may be interpreted by non-experienced specialists inadequately. Replacement or refinement of these kinds of items with evident markers applicable in clinical practice will improve research outcome and its practical benefits.

Author would like to temporarily end the discussion with phrases of Dr. Ronit Yoeli-Tlalim, recognized expert in history of Asian medicine from University of London "…cross-cultural approach to health and illness is central to public health and discussing perceptions of the body as culturally defined is not only important from a philosophical or historical point of view, but also has important practical ramifications …" [1].

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