Введение. Исследование врождённого иммунного ответа человека к клещевым инфекциям после подтверждённого факта укуса заражённым клещом на ранних, скрытных доклинических стадиях может показать реакцию организма человека и будет способствовать разработке новых способов ранней диагностики и профилактики трансмиссивных заболеваний.

Материалы и методы. Всего на заражённость вирусом клещевого энцефалита, B. burgdorferi sensu lato, A. phagocytophilum и E. chaffeensis / E. muris проанализировано 2821 образец крови от людей, пострадавших от присасывания иксодовых клещей. 1421 проба проанализирована на наличие антител к клещевым инфекциям. В заражённых пробах крови с помощью иммуноферментного анализа определена концентрация основных цитокинов.

Результаты и обсуждение. Установлена инфицированность людей после присасывания клещей: 3,2 % – РНК вируса клещевого энцефалита; 4,1 % – B. burgdorferi sensu lato; 0,45 % – A. phagocytophilum; 0,15 % – E. chaffeensis / E. muris. Показано, что бактериальные патогены, особенно B. burgdorferi sensu lato и A. phagocytophilum, индуцируют очень похожие профили врождённого иммунного ответа человека. Для этих микроорганизмов характерна активация IL-1α, IL-8 и IFN-α и подавление синтеза SOD, IL-1Ra и IL-17A. Инфекция ВКЭ на ранней стадии индуцирует своеобразный профиль цитокинового ответа у заражённого человека. Для вирусной инфекции характерно возрастание концентрации IL-1α, IL-8, IL-10, IL-17A, интерферона IFN-γ и, в отличие от бактериальных инфекций, SOD. Некоторое подавление синтеза указанных цитокинов отмечается только для рецепторного антагониста IL-1Ra.

Заключение. Показано, что иммунный ответ при клещевых инфекциях запускается сразу после заражения человека, и уже в первые 2–5 дней можно обнаружить изменения в концентрации основных цитокинов в плазме крови.

Ключевые слова: клещевые инфекции, клещевой энцефалит, иксодовый клещевой боррелиоз, моноцитарный эрлихиоз человека, гранулоцитарный анаплазмоз человека, образцы крови, цитокины

ПЕРВЫЕ РЕЗУЛЬТАТЫ ИЗУЧЕНИЯ «КЛЕЩЕВЫХ» ИНФЕКЦИЙ НА ДОКЛИНИЧЕСКОЙ СТАДИИ ИНФИЦИРОВАНИЯ ЧЕЛОВЕКА

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Currenty, viral and bacterial infections transmitted to humans by ticks are a serious health problem in many countries of Eurasia, including the Russian Federation, especially in the regions of the Siberian Federal District. An important feature of tick-borne infections is the polymorphism of clinical manifestations of the infection. However, most of the current data on the pathogenesis of tick-borne infections were obtained basing on studies of patients with severe clinical manifestations of infections.

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From the official statistical reports of the Federal Service for Supervision of Consumer Protection and Infectious and Parasitic Disease of the Russian Federation, it is known that approximately 3,000 cases of tick-borne encephalitis (TBE) and about 9,000 cases of Lyme disease (LD) are registered annually in Russia [4]. Recently two tick-borne infections less studied in our country – human granulocytic anaplasmosis and human monocytic ehrlichiosis – are recognized by physicians. The significance of the TBE and LD diseases is determined by possible fatalities, disability, transition to chronic form, and high cost of treatment. Tick attack rate is very high in endemic regions. Thus, in Irkutsk, the Centre for Diagnosis and Prevention of Tick-Borne Diseases (FBSC FHHRP) reports that approximately from 6.5 to 8.0 thousands of people bitten by ixodid ticks seek treatment annually.

There is a wide variety of clinical manifestations of tick-borne infections. Thus, up to 80 % of cases of TBE is followed by febrility without neurological manifestations or without any clinical symptoms at all. However, in 20–40 % a serious illness develops with the development of meningocerebral syndrome, and a possible chronicization of the infection. The incubation period of TBE is 7–21 days, while in the 2–4 days after infection the antigen of BSE and infectious virus are detected in the blood of infected people [2, 3, 14, 15, 16, 19, 20]. During the course of TBE in humans and in model animals, stimulation of elements of the innate immune response, in particular, interferons α and β (IFN-α, IFN- β), interferon gamma (IFN-γ), tumor necrosis factor (TNF-β) and interleukin 6 (IL-6) [1, 5, 23, 27].

For LD, there is also a wide variability in clinical manifestations, including feverish forms, severe damage of joints (lyme-arthritis), skin (acrodermatitis), and central nervous system (neuroborreliosis). There is a correlation between the species of B. burgdorferi sensu lato and the features of the course of the disease [7, 25, 26]. In patients with LD, the induction of cytokines IL-6, IL-8, IL-12, IL-18, IFN-γ, IFN-β and CXCL13 predominates [9, 17].

The causative agent of HGA – A. phagocytophilum – was opened in 1994. The infection is distributed across the entire territory of North America and Eurasia [10, 13, 21]. In the Russian Federation, A. phagocytophilum was detected in I. persulcatus ticks both in European [12] and in Asian [22; 24] parts of the country. Incidence of HGA has the highest rates in the US: up to 65 cases, with an average of 0.3–1.5 cases per 100 thousands of population [6]. The immune layer among the healthy population in the United States is between 0.5 and 3.5% [18], however, ranging from 9 to 36 % among the people exposed to tick bites. At the same time, most infections are asymptomatic [6, 11]. In the immunopathogenesis of HGA, the expression of IFNgamma is considered one of the limiting factors of the infection [8]. In Russia, the real epidemiological significance of HGA is not completely characterized.

In the literature, we have not found any studies devoted to the complex study of the earliest stages of tick-borne infections in the humans. The closest studies describe the course of infection in patients after the emergence of a full range of clinical symptoms of the infectious process. With another approach, applied in modern science, the course of infection in model systems – laboratory animals and cell cultures – has been studied. In this regard, the aim of the study is to evaluate the course of infection and the dynamics of the immune response at the earliest, subclinical stages in individuals infected through the bite of ixodid ticks.

**MATERIALS AND METHODS**

The samples of plasma and the leukocyte fraction of the blood of people who attended the Centre after tick bite during 2016 were used in the work. In total, 2,281 blood samples were tested for the infection with TBEV, B. burgdorferi sensu lato, A. phagocytophilum and E. chaffeensis / E. muris and 1/421 samples were examined in ELISA for the presence of antibodies to the abovementioned infections. Three main factors affecting the course and outcomes of tick-borne infections were studied: the pathogens themselves, the innate immunity of patients and the humoral immunity of patients after the bite of an infected tick. Each of these elements was examined both at the time of infection and in dynamics, which allowed to assess more fully their effect on the development of infection in the subclinical stage.

The ELISAtest “IFA TCA GT BBEV” (FGUP “NPO” Microgen”, Russia, Moscow) was used to detect the antigen of TBEV in blood samples. The nucleic acids of TBEV, B. burgdorferi sensu lato, A. phagocytophilum and E. chaffeensis / E. muris were detected using the multiplex real-time PCR kit “Multiprime” (Amplisens, Moscow). To confirm the fact of human infection, positive samples were verified either in analysis of ELISA / PCR, or in PCR with primers to specific fragments of the genome of the corresponding pathogens (Table 1).

Detection of immunoglobulins IgM and IgG for TBEV, B. burgdorferi sensu lato, A. phagocytophilum and E. chaffeensis / E. muris were produced with the help of ELISA test systems VektotBEV-IgM, VektotBEV-IgG, LymeBest-IgM and LymeBest-IgG (Vector Best, Novosibirsk), HGA-IgM, HGA-IgA-IgG, HME-IgM and HME-IgA-IgG (OOO Omnim, St. Petersburg) according to the manufacturers’ instructions.

The spectrum and balance of cytokines were assessed by ELISA test systems for quantifying the concentration of interleukins IL-1α, IL-1β, IL-4, IL-8, IL-10, IL-17A, IL-18, granulocyte colony stimulating factor G-CSF, tumor necrosis factor (TNF-α, interferon IFN-α and IFN-γ, superoxide dismutase SOD, and IL-1Ra interleukin-1 receptor antagonist. The test systems of OOO Tsitokin (St. Petersburg) were used, with the exception of the test system for estimating IL-18 interleukin (Vector-Best, Novosibirsk) according to the manufacturers’ instructions.

The results were presented as mean values; a standard deviation of the mean values was calculated to estimate the variability of the results. To analyze the results obtained, we used standard statistical methods, such as the Shapiro – Wilk test, the Mann – Whitney U-test, the Kruskall – Wallace criterion, the Student’s-t-test, and others.

**RESULTS AND DISCUSSION**

**Infection of people with pathogens of tick-borne infections after a tick bite**

Analysis of tick-borne pathogens in infected people showed that 3.2 % of people had TBEV antigen, 1.8 % had TBEV RNA, and 4.1 %, 0.45 % and 0.15 % had RNA/DNA of B. burgdorferi sensu lato, A. phagocytophilum and
**E. chaffeensis / E. muris**, respectively. Analysis of human blood samples for the presence of antibodies to tick-borne pathogens revealed the presence of IgM antibodies to the agents of the TBE, Lyme disease, HME and HGA in 16%, 10%, 14% and 11% of samples, respectively. IgG antibodies to these infections were detected in 4.8%, 30%, 12% and 6% of the population. Interestingly, in 85 cases (~2% of the affected), none of the trivial causative agents of tick-borne infections was detected, as were the antibodies to them. However, these patients developed obvious health disorders after the tick bite.

Based on the data obtained, a group of people with positive samples (herein after “test group”, 89 people) and a control group (45 people) were formed. These groups were used for further study of tick infections at the preclinical stage. In particular, the tests confirming the fact of infection, the characteristics of the genetic diversity of microorganisms, analyses of humoral and innate immunity were conducted. Independent confirmation of infection was obtained for 58 samples (65%) from the test group. All samples from the control group had significantly negative results on TBEV, **B. burgdorferi sensu lato**, **A. phagocytophilum** or **E. chaffeensis / E. muris**.

**Human humoral immune response at the preclinical stage of the infection**

Analysis of the humoral immune response in the test group showed that after the prevention of tick infections, 10 out of 58 people still produced antibodies to causative agents of TBEV (6 cases) or Lyme disease (4 cases). The dynamics of the humoral immune response in all cases corresponded to the previously described data. IgM antibodies were detected at 11–23 days after infection, while the production of IgG antibodies occurred at later stages of infection (30–51 days after the tick bite). There were no antibodies detected to the agents of HME or HGA. No antibodies were detected at the earliest stages of infection at 2–11 days after the tick bite as well. This indicates that none of the people surveyed in the foreseeable past was infected with TBEV, **B. burgdorferi sensu lato**, **A. phagocytophilum**, or **E. chaffeensis / E. muris**. Thus, it can be assumed that all observed responses of the human body are associated with the investigated case of infection, and not with previous contacts of the infected people with any of these pathogens.

**Innate immune response in humans at the preclinical stage of the infection**

Analysis of the spectrum of cytokines present in the blood of uninfected people showed that all the cytokines in the control group are present. However, the occurrence of each of them varied greatly. For example, interleukin IL-1β was found in only 3% of persons affected by the bite of an infected tick, IL-1R receptor antagonist IL-1R – in 17 %, whereas such cytokines as IFN-α, IFN-γ and SOD were expressed in all the examined people from the control group. A detailed description of the cytokine profiles of the control and test groups is given in Table 2.

In humans infected with TBEV at an early stage of infection, virtually all cytokines are also found, with the exception of IL-1β, which was not detected. However, the occurrence of some of them is significantly different in comparison with the control group. For example, IL-6 (in 77% of people infected with TBEV, compared to 47 of the control group), IL-17A, IL-18 and IL-1RA are significantly more often detected in blood samples from infected humans (Table 2). At the same time, IL-1x is less common in the test group than in the control group (30% vs. 53%).

For **B. burgdorferi sensu lato** infection, the overall spectrum of cytokines is similar to that of a subset of people infected with TBEV, but among these patients no expression of...
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The IL-1Ra receptor antagonist has been found. There was a significant increase in the proportion of people with interleukins IL-1α, IL-6 and IL-18. At the same time, the proportion of patients with G-CSF and TNF-α present in the blood is slightly reduced relative to the control group (Table 2).

For confirmed cases of human infection by *A. phagocytophilum* and *E. chaffeensis / E. muris*, the quantitative characteristics of expression of the components of the innate immune response is given.

<table>
<thead>
<tr>
<th></th>
<th>IL-1alpha</th>
<th>IL-1beta</th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-17A</th>
<th>IL-18</th>
<th>G-CSF</th>
<th>TNF-alpha</th>
<th>IFN-alpha</th>
<th>IFN-gamma</th>
<th>SOD</th>
<th>IL-1Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBEV-infected, %</td>
<td>30</td>
<td>0</td>
<td>48,5</td>
<td>77</td>
<td>77</td>
<td>70</td>
<td>76</td>
<td>30</td>
<td>54,5</td>
<td>82</td>
<td>42</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B. burgdorferi s.l.-infected, %</td>
<td>100</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Control group, %</td>
<td>53</td>
<td>3</td>
<td>53</td>
<td>47</td>
<td>80</td>
<td>76</td>
<td>5,5</td>
<td>30,5</td>
<td>72</td>
<td>42</td>
<td>100</td>
<td>100</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 2

Occurrence of different cytokines in people infected with TBEV, B. burgdorferi s.l., and in control group (uninfected people)

![Graphs showing cytokine levels](image)

**Fig. 1.** Changes in cytokine levels after the infection with different tick-borne pathogens at subclinical stage of infection. The changes in comparison to control group (uninfected people) are shown.
When considering the quantitative characteristics of the innate immune response in the studied groups, it turned out that in all cases, the data obtained within the samples are characterized by high variability. However, even in this case, there are significant differences in the levels of cytokine expression in the groups of infected and uninfected patients (Fig. 1).

Thus, in TBEV infection, an increase in the concentration of IL-1α, IL-8, IL-10, IL-17A and interferon IFN-γ can be noted. Some inhibition of synthesis is noted for the IL-1Ra receptor antagonist. The greatest changes are observed in the level of SOD expression, the concentration of which increases almost threefold (from 61,000 to 172,000 pg/ml). The infection of B. burgdorferi sensu lato, on the contrary, leads to inhibition of SOD synthesis as well as IL-1Ra, while the concentration of IL-1α slightly increases. There is also a decrease in the expression level of IL-1ra, while the concentration of IL-1α slightly increases. There is noted only for the receptor antagonist IL-1Ra.

To bacterial infections, SOD. Some suppression of synthesis of IL-1α, IL-8, IL-10, IL-17A, interferon IFN-γ and, in contrast to viral infections, SOD. Some suppression of synthesis is noted only for the receptor antagonist IL-1Ra.

CONCLUSION

For the first time, a number of fundamental features of tick-borne infections were revealed, which must be taken into account when improving the measures of urgent diagnostics and prevention of tick-borne diseases. We found that only 65% of cases of human infection with TBEV, B. burgdorferi sensu lato, A. phagocytophilum and E. chaffeensis / E. muris is confirmed by an independent method. This may indicate the overdiagnosis of microorganisms in the screening test, for example, due to nonspecific reactions, especially when detecting an antigen of TBEV in plasma and human serum. The detection of specific antibodies to tick-borne pathogens after the prevention procedures (within 96 hours after sucking of an infected tick) indicates an important process of triggering an immune response in humans in the early stages of the preclinical stage. In our opinion, this is a sign of the active role of elements of innate immunity which are activated within a few hours after the infection event.

When assessing the cytokine profile of infected people at the preclinical stage, a number of fundamental features of the course of infection were revealed. Thus, it has been shown that bacterial pathogens, especially B. burgdorferi sensu lato and A. phagocytophilum induce very similar profiles of the innate human immune response. These microorganisms are characterized by activation of IL-1α, IL-8 and IFN-α and suppression of the synthesis of SOD, IL-1Ra and IL-17A. Infection of BSE at an early stage induces a peculiar profile of the cytokine response in an infected person. A viral infection is characterized by an increase in the concentration of IL-1α, IL-8, IL-10, IL-17A, interferon IFN-γ and, in contrast to bacterial infections, SOD. Some suppression of synthesis is noted only for the receptor antagonist IL-1Ra.

Thus, we showed that the immune response is triggered immediately after human infection, and already on the second or fifth day, changes in the expression levels of the main cytokines can be detected. The data obtained suggest that the evaluation of the intensity of the innate immune response after the bite of an infected tick can significantly increase the accuracy of the risk assessment of human infection with tick infections.

The study was carried out using the equipment of the Center for Collective Use “PCR-Diagnostics” of the FBSC FHHRP.

REFERENCES