University of South Bohemia
Faculty of Science
Department of Parasitology

Ph.D. Thesis

Interactions among *Ixodes ricinus* tick saliva, murine dendritic cells and the tick-borne encephalitis virus

Anna Skallová

Supervisor: Doc. RNDr. Jan Kopecký, CSc.

Laboratory of Vector-Host Interactions,
Institute of Parasitology,
Biology Centre, ASCR

České Budějovice, 2009

Skallová A., 2009: Interactions among *Ixodes ricinus* tick saliva, murine dendritic cells, and tick-borne encephalitis virus. Ph.D. thesis, in English – 32 pp. + appendices (32 pp.), Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation: Dendritic cells are among the first cells that encounter salivary antigens of blood-feeding arthropods, and are also supposed to be the early targets of tick-borne encephalitis virus, an important human pathogen naturally delivered into host skin via a bite of an infected tick. This thesis has generally focused on studying the impact of tick saliva on dendritic cell biology, and, subsequently, effects of infection with two different tick-borne encephalitis virus strains on dendritic cell maturation, cytokine production, T cell activation and apoptosis in the presence or absence of the tick saliva.

Financial support: This work was supported by Grant 524/05/0811 from the Grant Agency of the Czech Republic, by the project of the Basic Research Centre LC06009 from the Czech Ministry of Education, by Grant IAA600960811 from the Grant Agency of the ASCR, and by the research project of Institute of Parasitology Z60220518.

I hereby declare that I worked out this Ph.D. thesis on my own, or in collaboration with the co-authors of the presented papers and manuscript, and only using the cited literature.

I declare that in accordance with the Czech legal code § 47b law No. 111/1998 in its valid version, I consent to the publication of my Ph.D. thesis (in an edition made by removing marked parts archived by the Faculty of Science) in an electronic way in the public access to the STAG database run by the University of South Bohemia in České Budějovice on its web pages.

České Budějovice, 2009 Anna Skallová

Acknowledgements

I would like to thank Doc. RNDr. Jan Kopecký, CSc., my supervisor in České Budějovice, especially for buying me every antibody I had wished (although some of them didn't promise to help me get many interesting results). I'd also like to thank the tick fans from the Laboratory of Vector-Host Interactions, particularly my co-inhabitants in our shocking green study.

My special thanks belong to Giando Iezzi, the most enthusiastic scientist I have ever met. Giando often sacrificed her time to my technical and intellectual growth (although she wasn't really interested in the tick research before I came to Zurich) and, moreover, elucidated me, how important is the role of ice cream in research. My thanks also belong to the other members of Manfred Kopf's lab; it was a pleasure for me to work with them during the few weeks of my stay in Switzerland.

Kačka Černá, Ivka Schödelbauerová and Pafka Šorfová deserve my great thanks for keeping me sane. Indeed, it was Kačka, who has convinced me that throwing samples out of the window is not the best way how to get promising results.

I am also much obliged to Zdeněk Cimburek, the flow cytometry guru from the Institute of Microbiology in Prague, who always tried to acquire some valuable data from all of the samples I brought (even the half-dead and over-labelled).

Last but not least, I would like to thank my parents and my Feďa for their love, support, and endless patience. I promise you that there won't be any more dramatic flow cytometry trips to Prague (no more need to worry about your car, dad!).

The thesis is based on following papers:

Skallová A, Iezzi G, Ampenberger F, Kopf M, Kopecký J. Tick saliva inhibits dendritic cell migration, maturation, and function while promoting development of Th2 responses. J Immunol 2008, 180:6186-6192.

Skallová A, Cimburek Z, Iezzi G, Kopecký J. *Ixodes ricinus* tick saliva modulates tick-borne encephalitis virus infection of dendritic cells. Manuscript ready for submission to the Journal of Infectious Diseases.

Růžek D, Salát J, Palus M, Gritsun TS, Gould EA, Dyková I, **Skallová** A, Jelínek J, Kopecký J, Grubhoffer L. $CD8^+$ T-cells mediate immunopathology in tick-borne encephalitis. Virology 2009, 384:1-6.

Papers not included in the thesis:

Horká H, Černá-Kýčková K, **Skallová A**, Kopecký J. Tick saliva affects both proliferation and distribution of *Borrelia burgdorferi* spirochetes in mouse organs and increases transmission of spirochetes to ticks. Int J Med Microbiol 2009, (in press).

Novotná M, Havlícek J, Smith AP, Kolbeková P, **Skallová A**, Klose J, Gasová Z, Písacka M, Sechovská M, Flegr J. *Toxoplasma* and reaction time: role of toxoplasmosis in the origin, preservation and geographical distribution of Rh blood group polymorphism. Parasitology 2008, 135:1253-1261.

Hodková H, Kolbeková P, **Skallová A**, Lindová J, Flegr J. Higher perceived dominance in *Toxoplasma* infected men – a new evidence for role of increased level of testosterone in toxoplasmosis-associated changes in human behaviour. Neuro Endocrinol Lett 2007, 28:110-114.

Lindová J, Novotná M, Havlicek J, Jozifková E, **Skallová A**, Kolbeková P, Hodny Z, Kodym P, Flegr J. Gender differences in behavioural changes induced by latent toxoplasmosis. Int J Parasitol 2006, 36:1485-1492.

Skallová A, Kodym P, Frynta D, Flegr J. The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. Parasitology 2006, 133:525-535.

Skallová A, Novotná M, Kolbeková P, Gašová Z, Veselý V, Sechovská M, Flegr J. Decreased level of novelty seeking in blood donors infected with *Toxoplasma*. Neuro Endocrinol Lett 2005, 26:480-486.

Abbreviations

APC	antigen-presenting cell
BIP	B cell inhibitory protein
CCR	CC chemokine receptor
ConA	concanavalin A
DC	dendritic cell
HCMV	human cytomegalovirus
HIV	human immunodeficiency virus
HRF	histamine release factor
ICAM	intercellular adhesive molecule
IFN	interferon
Ig	immunoglobulin
IGBP	immunoglobulin-binding protein
IL	interleukin
IRAC	*Ixodes ricinus* anticomplement
ISAC	*Ixodes scapularis* anticomplement
LPS	lipopolysaccharide
LTA	lipoteichoic acid
MHC	major histocompatibility complex
MHCI	MHC Class I
MHCII	MHC Class II
MIP	macrophage inflammatory protein
MV	measles virus
NK	natural killer
PD-1	programmed death 1
PGE2	prostaglandin E2
SAT	saliva-activated transmission
SGE	salivary gland extract
TBE	tick-borne encephalitis
TBEV	tick-borne encephalitis virus
TCR	T cell receptor
TGF	transforming growth factor
Th	helper T cell
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T cell
WHO	World Health Organization

Table of contents

1. Introduction

1.1 Tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV), in compliance with the recent scheme (Thiel et al., 2005) a member of the genus *Flavivirus* within the family Flaviviridae, belongs according to the World Health Organization to the most important arthropod-borne viruses in Europe causing severe human infections (WHO, 2004). Tick-borne encephalitis (TBE) occurs in humans following the bite of an infected tick (*Ixodes ricinus* in Europe, *I. persulcatus* in Asia) and, rarely, after the ingestion of unpasteurized milk from infected domestic animals (Grešíková et al., 1975; Dumpis et al., 1999). The causative agent of TBE was first isolated in eastern Russia in 1937 (Zilber, 1939); however, the disease was already described as a clinical entity in Austria in 1931 (Schneider, 1931).

Icosahedral enveloped TBE virions have an average diameter of 50 nm. The electron dense core contains an 11 kb long single stranded RNA genome of positive polarity, which encodes large polypeptide of approximately 3400 amino-acids. The viral polypeptide is cleaved into three structural (capside, membrane and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) by cellular and viral proteases (Heinz, 1986; Lindenbach and Rice, 2001). The secreted form of the NS1 protein has been reported to induce protective immune responses against flaviviruses in general (Gould et al., 1986; Jacobs et al., 1992; Timofeev et al., 1998). Other non-structural proteins have been reported to provide virus-specific serine protease activity as well as RNA-dependent RNA polymerase machinery (Lindenbach and Rice, 2001) and, additionally, seem also to modulate host immune responses by inhibition of interferon signalling (Best et al., 2005).

The endemic area of TBEV spreads from Western Europe to Far Eastern Russia and the virus is taxonomically classified into three subtypes, namely European, Siberian and Far Eastern (Ecker et al., 1999). In Europe, approximately 4,000 cases of human TBE are annually reported. In addition, up to 8,000 cases are reported each year from Russia (WHO, 2004). The incubation period of TBE generally lasts 7-14 days, whereas the disease severity and clinical symptoms are assumed to be associated with the TBEV subtype (Votiakov et al., 1978; Gritsun et al., 2003). European viruses typically cause biphasic infections with the first stage accompanied by influenza-like symptoms including

fever, headache, fatigue and general malaise (Mickiené et al., 2001). During the second stage, 20–30% of patients develop neurological symptoms ranging from mild meningitis to severe encephalitis (Duniewicz et al., 1975; Kaiser et al., 1999; Mickiené et al., 2001). Case fatality rates usually vary from 1 to 5% (Grešíková and Kaluzová, 1997; Herzig et al., 2002). Infections with Far Eastern viruses are known to be the most severe, with case fatality rates as high as 20-60%. Exceptionally, Far Eastern viruses have been reported to bring on fatal hemorrhagic syndrome typical for infections caused by certain mosquito-borne flaviviruses, such as Yellow fever (Ternovoi et al., 2003). On the contrary, Siberian viruses characteristically cause chronic infections with case fatality rates rarely exceeding 8% (Pogodina et al., 1981a,b; Fokina et al., 1982; Gritsun et al., 2003).

As mentioned above, TBEV is naturally delivered into the host skin during the prolonged blood sucking of an infected tick. It is supposed that in the early phase of infection, the virus replicates within the dermis and subsequently in the skin draining lymph nodes (Albrecht, 1968). However, the way the virus enters the lymph node remains unknown. One possibility might be that free virus particles reach the lymph nodes through lymphatic vessels. Alternatively, virus could be carried to the regional lymph nodes by migratory dendritic cells (DCs) (Masurier et al., 1998; Pohl et al., 2007). Virus replication in regional lymph nodes is followed by its spread to other tissues including the central nervous system (Albrecht, 1968).

Development of systemic viraemia is not necessary for virus transmission. Surprisingly, TBEV transmission has been reported to be even more efficient among ticks co-feeding on a non-viraemic host in comparison to a host with high viraemia (Labuda et al., 1993a). Moreover, transmission among co-feeding ticks is possible also on TBEV-immune hosts producing potent anti-viral antibodies (Labuda et al., 1997). At the infestation site, the virus has been shown to replicate in resident DCs as well as macrophages and neutrophils recruited from blood. Whereas only the skin at tick bite-sites was infected, but not the uninfested skin, it has been suggested that TBEV is carried among co-feeding ticks by immune cells migrating from one infestation site to another. This data suggests that viremia may be a product of TBEV transmission rather than a prerequisite (Labuda et al., 1996).

1.2 Immune response to tick infestation

During co-evolution with their hosts, ticks similarly to other blood-feeding arthropods had to evolve mechanisms necessary to overcome host haemostatic and immune systems to successfully complete their meal. These mechanisms are important especially for ticks, which require a long-term contact with the host (usually 3–10 days). Indeed, tick saliva has been shown to contain a wide range of bioactive molecules with vasodilatory, anti-haemostatic, and immunomodulatory activities (Ribeiro et al., 1985; Wikel, 1996). Despite this fact, tick feeding may induce a complex immune response involving various components of the immune system (Wikel et al., 1996).

During the first infestation of a naïve host, the tick attachment site is infiltrated primarily by mononuclear cells and neutrophils, followed by mast cells, basophils and eosinophils (Brown et al., 1984; Brown, 1985). It is supposed that tick saliva antigens are captured by Langerhans cells in the skin and subsequently presented to T lymphocytes in a draining lymph node (Allen et al., 1979; Nithiuthai and Allen, 1985).

As a result of repeated infestations, some hosts are able to develop resistance to tick feeding. Such acquired host resistance is manifested by reduced engorgement weights of ticks, longer feeding periods, decreased production and viability of ova, impaired molting and tick death (Wikel, 1996). Strong immune response of guinea pigs repeatedly infested by *Dermacentor variabilis* larvae was described as early as in 1939 (Trager, 1939). Since that time, immunity to ticks has been observed in many other species including rabbits, bovines and wild and laboratory rodents (Bowessidjaou et al., 1977; Brown et al., 1984; denHollander and Allen, 1985; Dizij and Kurtenbach, 1995). Immune response to repeated tick infestations is characterized by marked infiltration of basophils and eosinophils to the tick bite-site. Thus, with regard to the predominance of basophils this form of Th1 mediated delayed type hypersensitivity is known as cutaneous basophil hypersensitivity (Richerson et al., 1970; Singh and Girschick, 2003). However, it is supposed that host-acquired resistance to tick infestation involves much more regulatory and effector components of the immune system including complement, antigen-presenting cells (APCs,), B and T lymphocytes, cytokines, circulatory and haemocytotrophic antibodies, as well as granulocytes together with the bioactive mediators released by these cells (Wikel, 1999; Singh and Girschick, 2003).

Effects of tick feeding on cytokine expression patterns in the skin or skin draining lymph nodes have been already described by many authors. Mbow et al. (1994) have

reported a strong expression of IFN-γ and IL-2 mRNA in the skin of BALB/c mice repeatedly infested by *I. ricinus* nymphs. Cytokine expression in the skin during the primary infestation was very low; however, IFN-γ and IL-2 mRNA positive cells were detected in the lymph nodes. Surprisingly, the IFN-γ and IL-2 expressing cells prevailed over the IL-4 mRNA positive cells even in repeatedly infested mice. On the contrary, Ganapamo et al. (1995) have observed high production of IL-4 and low production of IFN-γ in ConA-stimulated lymph node cells isolated from both, once as well as repeatedly infested BALB/c mice. Similar results have been also reported by Schoeler et al. (1999) in Con A stimulated mouse splenocytes, or by Mejri et al. (2001) after restimulation of the isolated lymph node cells with the tick saliva or salivary gland extract (SGE). With regard to these results, it is supposed that development of Th2 subsets, characterized by production of high levels of IL-4, and a corresponding decrease of Th1 inflammatory responses are mediated by immunomodulatory molecules contained in the tick saliva (Schoeler et al., 1999; Gillespie et al., 2000; Brossard and Wikel, 2004).

1.3 Immunomodulatory activities of the tick saliva

As mentioned above, ticks have to circumvent not only the haemostatic, but also the immune system of their hosts to complete their blood meal successfully. Therefore, tick saliva contains besides anticoagulants, anti-platelet factors and vasodilators a wide spectrum of molecules with various immunomodulatory activities that affect both, innate as well as adaptive immune responses (Ribeiro et al., 1985; Wikel, 1996; Valenzuela, 2004; Titus et al., 2006).

The first immunomodulatory effect of the tick saliva, namely the kininase activity, was described as early as in 1985 by Ribeiro et al. The molecule responsible for mentioned proteolytical cleavage of bradykinin, a mediator of pain and oedema formation, was later described as metallodipeptidylcarboxypeptidase (Ribeiro and Mather, 1998).

Not surprisingly, ticks have also evolved mechanisms how to inhibit other potent inflammatory mediator causing oedema, histamine, which is released from mast cells and basophils usually via an IgE-dependent mechanism (Beneditt et al., 1955; Shim and Oh, 2008). Indeed, a high-affinity histamine-binding protein has been identified in *Rhipicephalus appendiculatus* tick saliva (Paesen et al., 1999). Subsequently, proteins homologous to *R. appendiculatus* histamine-binding protein have been described in the salivary glands of the tick *I. scapularis* (Valenzuela et al., 2002) and *Amblyoma*

americanum (Aljamali et al., 2003). Additionally, a similar protein with two binding sites, one for histamine and the other for serotonin, has been isolated from the salivary glands of the tick *Dermacentor reticulatus* (Sangamnatdej et al., 2002). On the contrary, *D. variabilis* tick saliva have been reported to contain a protein homologous to human histamine release factor (HRF), which induces histamine secretion from basophils and mast cells (Mulenga et al., 2003). Similar HRF homologs have also been identified in the saliva of *Boophilus microplus* and *A. americanum* (Mulenga and Azad, 2005).

Another important part of the inflammatory immune response and pathogen evasion is complement cascade activation, which results in anaphylatoxin production, deposition of opsonins on cell surfaces and membrane attack complex formation (Gasque, 2004). Ribeiro and Spielman (1986) have shown that *I. scapularis* saliva antagonizes anaphylatoxin C3a, an important activator of mast cells and basophils. Moreover, *I. scapularis* saliva has been reported to impair the alternative pathway of complement activation by inhibiting binding of C3b and C5b components to cell surfaces (Ribeiro, 1987). In more recent studies, different anti-complement molecules have been identified in the tick saliva, including ISAC (Valenzuela et al., 2000) or Salp20 (Tyson et al., 2007) in *I. scapularis*, and IRAC I and IRAC II in *I. ricinus* (Schroeder et al., 2007; Daix et al., 2007).

Tick saliva has also been shown to partly control cell infiltration into tick bite-site by inhibiting activity of various chemokines. Hajnická et al. (2001) described IL-8 binding activity of SGE from several tick species including *D. reticulatus*, *A. americanum, R. appendiculatus, Haemophysalis inermis* and *I. ricinus*. Moreover, SGEs markedly inhibited binding of IL-8 to its receptors on human granuolocytes. In a following study, MIP-1α binding activity has been described in SGEs of *D. reticulatus* and *A. americanum* (Hajnická et al., 2005). IL-8 is a potent chemo-attractant mainly for neutrophils, whilst MIP-1α is supposed to exert chemotactic activities toward a variety of immune cells, including granulocytes, monocytes, lymphocytes, macrophages and bone marrow-derived DCs (Schall et al., 1993; Bennouna et al., 2003). In a recent study focused on *R. sanguineus* salivary immunomodulators, the protein responsible for MIP-1α binding has been identified and described as Evasin-1 (Frauenschuh et al., 2007). Other tick saliva-derived Evasins have been subsequently identified by Déruaz et al. (2008). Additionally, *I. scapularis* tick-borne sialostatin L has been shown to inhibit neutrophil recruitment into a carrageenan-induced site of inflammation. However, the exact mechanism of this effect has not been evaluated so far (Kotsyfakis et al., 2006). Similarly, recently described

leukotriene-binding *I. ricinus* salivary protein Ir-LBP has been reported to inhibit neutrophil chemotaxis in vitro and in vivo (Beaufays et al., 2008).

Kubeš et al. (1994) described a suppressive effect of *D. reticulatus* SGE on human natural killer (NK) cell cytotoxic activity. Similar, although not so pronounced inhibition of NK cells has been detected also for SGEs derived from *A. variegatum* and *H. inermis*, but not for SGEs from *I. ricinus* and *R. appendiculatus* (Kubeš et al., 2002). However, NK-inhibitory effect of *I. ricinus* SGE has been formerly reported by Kopecký and Kuthejlová (1998).

According to Urioste et al. (1994), exposure to *I. scapularis* saliva markedly inhibited nitric oxide (NO) production by LPS-stimulated macrophages. Similarly, saliva from *R. sanguineus* significantly inhibited NO production by IFN-γ-activated intraperitoneal macrophages infected with *T. cruzi*. Moreover, this effect was markedly correlated with the impairment of intracellular killing of parasites by macrophages (Ferreira and Silva, 1998). In agreement with these observations, Kuthejlová et al. (2001) described an inhibitory effect of *I. ricinus* SGE on intracellular killing of *Borrelia afzelii* spirochetes by murine macrophages. SGE treated macrophages have been also shown to produce lower levels of NO and superoxide in response to *Borrelia* spirochetes. Additionally, *I. ricinus* SGE has been observed to diminish phagocytosis and TNF-α production in macrophages activated by *B. afzelii* and IFN-γ (Kýčková and Kopecký, 2006). Comparable inhibition of inflammatory cytokine production in macrophages or macrophage-like cell line has been reported for SGEs derived from *D. andersoni* (Ramachandra and Wikel, 1992) and *R. appendiculatus* (Gwakisa et al., 2001), respectively.

In accordance with studies focused on macrophages, *I. scapularis* tick saliva has been observed to diminish superoxide secretion and phagocytic capacity of peritoneal rat neutrophils (Ribeiro et al., 1990). Montgomery et al. (2004) have shown that *I. scapularis* saliva markedly reduced expression of neutrophil β2-integrins, which resulted in decreased attachment and spreading of activated cells. Additionally, the ability of neutrophils to bind *Borrelia* spirochetes was also reduced in the presence of the tick saliva.

Many authors mention the impact of tick saliva and/or SGE on mitogen-stimulated splenocytes, peripheral blood lymphocytes or purified T lymphocytes in vitro (Urioste et al., 1994; Ferreira and Silva, 1998; Kovář et al., 2001; Mejri et al., 2002; Turni et al. 2004). Bergman et al. (1998; 2000) have described a 36 kDa soluble protein in the saliva of *D. andersoni*, which has been shown to suppress proliferative response of murine

splenocytes to ConA. Similarly, Leboulle et al. (2002) have observed significant decrease in proliferation of ConA-stimulated T cells in the presence of *I. ricinus* salivary protein described as Iris. More recently, the *I. scapularis* salivary protein Salp15 has been reported to inhibit T lymphocyte activation and proliferation via specific interaction with CD4 coreceptor and subsequent inhibition of T cell signalling pathways (Anguita et al., 2002; Garg et al., 2006; Juncadella et al., 2007).

I. ricinus SGE has also been observed to induce hyporesponsiveness of B cells in response to mitogens (Hannier et al., 2003). In a later study, a molecule responsible for this suppressive activity was characterized and described as B cell inhibitory protein (BIP) (Hannier et al., 2004). Not only production, but also effectivity of antibodies has been shown to be affected by the tick saliva. Wang and Nuttall (1994; 1995) have detected immunoglobulin-binding proteins (IGBPs) in salivary glands and haemolymph of *R. appendiculatus, A. variegatum* and *I. hexagonus.* It is supposed that IgG molecules contained in bloodmeal are recognised and bound by IGBPs, transported via haemolymph into salivary glands and excreted with the saliva back into the tick feeding pool. It has been suggested that this mechanism may represent a self-defence system of the tick to protect itself against the potentially harmful effects of immunoglobulins from immune hosts. Moreover, excreted IgG could compete for Fc receptors on mast cells and basophils, consequently delaying or diminishing antibody-dependent immune response leading to tick rejection (Wang and Nuttal, 1999).

As indicated above, tick saliva and SGE have been reported to diminish the production of Th1-related cytokines and increase the production of Th2-related cytokines. Indeed, it has been shown that T cells exposed to *D. andersoni* SGE produced lower levels of IFN-γ and IL-2 in response to LPS (Ramachandra and Wikel, 1992). Similarly, lower levels of IFN-γ and higher levels of IL-10 in LPS-activated splenocyte cultures incubated with *I. ricinus* SGE have been reported by Kopecký et al. (1999). Fuchsberger et al. (1995) have observed significant decrease in mRNA expression of IFN-α, TNF-α, IL-1α, IL-1β, IL-5, IL-6, IL-7 and IL-8 by human peripheral blood leucocytes treated with LPS and *R. appendiculatus* SGE. Additionally, Ferreira and Silva (1999) have shown an enhancement of TGF-β and decrease in IL-12 production by murine splenocytes exposed to *R. sanguineus* tick saliva. Part of these effects may be mediated by IL-2-binding protein, which has been identified in the saliva of *I. scapularis* by Gillespie et al. (2001). Nevertheless, it has been discussed recently that tick-induced Th2 polarization can also be mediated by DCs (Mejri and Brossard, 2007; Sá-Nunes et al., 2007; Hovius et al., 2008b).

Indeed, Cavassani et al. (2005) have demonstrated inhibited differentiation of bone marrow-derived DCs in the presence of *R. sanguineus* tick saliva. Furthermore, tick saliva impaired LPS-induced DC maturation, IL-12 production and consequently DC-mediated stimulation of cytokine production by specific T cells. In a later study, *R. sanguineus* saliva has been shown to inhibit chemotaxis of immature bone marrow-derived DCs by down-regulating CC chemokine receptor CCR5 (Oliveira et al., 2008). Mejri and Brossard (2007) suggested that murine spleen-derived DCs pulsed with *I. ricinus* saliva may favour the Th2 differentiation of naïve $CD4^+$ T cells. Sá-Nunes et al. (2007) have identified *I. scapularis* salivary prostaglandin E2 (PGE2) as the molecule responsible for inhibition of IL-12 and TNF-α production by bone marrow-derived DCs upon TLR-2, TLR-4 and TLR-9 ligation. Tick saliva or PGE2 also reduced the ability of DCs to stimulate antigen-specific $CD4^+$ T cell proliferation. However, PGE2 is undoubtedly not the only DC inhibitory molecule in tick saliva. The *I. scapularis* salivary protein Salp15 has been shown to interact with the C-type lectin on human DCs, namely the DC-SIGN, which results in the inhibition of IL-6, IL-12, and TNF-α production. Results also show suppression of T cell-stimulatory capacity of LTA-, LPS-, or *B. burgdorferi*-activated DCs (Hovius et al., 2008b). Considering that antigen-presenting cells, especially DCs, are known to be major players in initiation and fine-tuning of the adaptive immune response, alteration of their function could be a key mechanism used by the tick to circumvent the host immune system.

1.4 Saliva-activated transmission (SAT)

It is supposed that immunomodulatory activities of the tick saliva can be exploited by tick-borne pathogens to facilitate their transmission and replication in the host. Such promotion of pathogen transmission through effects of salivary molecules has been termed saliva-activated transmission (SAT). Until now, only *I. scapularis* salivary protein Salp15 and its *I. ricinus*-derived homolog Salp15 Iric-1 have been identified as an unambiguous direct SAT factors facilitating *Borrelia* transmission into the host. Both of these molecules have been reported to bind to *B. burgdorferi* outer surface protein (Osp) C, protecting the spirochete from antibody-mediated killing (Ramamoorthi et al., 2005; Hovius et al., 2008a).

The term SAT was first used to describe the enhancing effect of *R. appendiculatus* tick SGE on Thogoto virus transmission (Nuttall and Jones, 1991). Since then, the evidence of SAT has been reported in many pathogen-vector pairs, including *La Crosse*

virus and *Aedes triseriatus* mosquito (Osorio et al., 1996), *Leishmania major* and *Phlebotomus papatasi* sandfly (Belkaid et al., 1998) or *Plasmodium berghei* and its vector, *Anopheles stephensi* mosquito (Vaughan et al., 1999). Similarly, SGEs derived from ixodid ticks have been shown to enhance infectivity in the bacteria *Borrelia afzelii* (Pechová et al. 2002), *B. burgdorferi* sensu stricto, *B. luisitaniae* (Zeidner et al., 2002) and *Francisella tularensis* (Kročová et al., 2003). Conversely, mice exposure to repeated infestation with pathogen-free *I. scapularis* nymphs markedly reduced following transmission of *B. burgdorferi* spirochaetes by infected ticks (Wikel et al., 1997). A comparable effect has been observed by Nazario et al. (1998) in guinea-pigs repeatedly exposed to *I. scapularis* nymphs or larvae. Therefore, it is supposed that immunity to tick feeding may prevent to a certain degree pathogen transmission via a tick bite.

Similar direct evidence of SAT with TBEV has been first reported in 1991 by Alekseev et al. According to Labuda et al. (1993b), SGEs derived from partially fed *I. ricinus*, *D. reticulatus* and *R. appendiculatus* adult females significantly enhanced the probability of TBEV transmission from infected guinea-pigs to feeding ticks. Simultaneously, development of viraemia was observed in markedly higher proportion of guinea-pigs inoculated with virus and SGE, in comparison with guinea-pigs inoculated with virus alone. However, systemic viraemia is not necessary for virus transmission. Indeed, efficient non-systemic transmission between infected and uninfected ticks co-feeding on the same host has been demonstrated as early as 1987 using Thogoto virus as a model pathogen (Jones et al., 1987). Such indirect evidence of SAT was later confirmed also by Labuda et al. (1993a) for TBEV. Moreover, the greatest number of infected ticks was obtained from naturally susceptible host species, *Apodemus flavicolis*, with undetectable or very low levels of viraemia. On the contrary, virus transmission between infected and uninfected ticks was less apparent on viraemic host *Cletrionomis glareolus*. In their following study, Labuda et al. (1997) have demonstrated that even hosts with neutralizing antibodies to TBEV are still able to support virus transmission between infected and uninfected ticks provided they feed close together. In case of the non-viraemic transmission, TBEV has only been detected in the skin at site of the tick bite, but not in the uninfested skin. This data indicates that the infested skin may be essential for TBEV replication early after virus transmission by ticks (Labuda et al., 1996).

The role of salivary components in pathogen transmission has been extensively studied for almost 30 years. However, searching for SAT factors is still on going, due to the possibility that vaccines targeted against certain salivary molecules might be effective

in blocking the transmission of tick-borne pathogens during tick feeding (Nuttall and Labuda, 2004).

1.5 Dendritic cells – the key targets of tick salivary bioactive molecules and TBEV?

Dendritic cells are widely accepted as the most potent APCs in the immune system. As well as the other white blood cells, they are generated from haematopoietic stem cells; however, it is still discussed, if different specialized DC subtypes are products of separate progenitor lineages or if the functional and phenotypic differences in DC subtypes predominantly depend on local environmental signals (Shortman and Liu, 2002). As professional APCs, DCs are specialized for the uptake, processing and presentation of self as well as foreign antigens to T lymphocytes (Steinman, 1991). Immature migratory DCs create a network in peripheral tissues and act as sensors continuously sampling surrounding antigens. Upon sensing of "danger signals" including microbial products or signals from damaged tissues, DCs become activated and migrate to regional lymph nodes to initiate specific immune responses (Matzinger, 2002). Such mature DCs loose their ability to capture new antigens, but they are very efficient at presenting the antigens they already captured (Wilson et al., 2003). Short peptides derived from the processed antigens are presented by major histocompatibility complex (MHC) molecules on the DC surface. In general, intracellular antigens are presented by MHC Class I (MHC I) molecules to interact with TCR of cognate $CD8^+$ T cells, whilst exogenous antigens are degraded in endosomal compartments, bound by MHC Class II (MHC II) molecules and presented to cognate $CD4^+$ T cells (Cresswell, 2005). Nevertheless, a process called cross-presentation, when certain exogenous viral, bacterial, or tumor-associated antigens are presented by MHCI molecules in order to generate cytotoxic $CD8^+$ T cell responses, has been additionally described in DCs (Yewdell et al., 1999; Heath and Carbone, 2001; Basha et al., 2008). Nonprotein antigens are known to be presented by CD1 molecules (Gumperz and Brenner, 2001). Activated DCs also upregulate co-stimulatory molecules, such as B7-1 (CD80) and B7-2 (CD86), which are required for effective stimulation of specific T cells. Indeed, T cells that interact with immature DCs remain unresponsive or tolerised, rather than activated. This mechanism is crucial for maintenance of peripheral tolerance, since in the steady-state DCs continuously present self-antigens on their surface and it was shown that not all of the self-reactive T cells are eliminated during development in the thymus (Steinman et al., 2000; Heath and Carbone, 2001).

DCs play a crucial role not only in initiating, but also in fine-tuning the adaptive immune responses, since they mediate polarization of naïve specific $CD4^+$ T lymphocytes to regulatory T cells (Treg) or to effector T helper type 1 (Th1), type 2 (Th2) and type 17 (Th17) lineages. The phenotype of a polarized T cell is determined by the complex of factors, including the dominant cytokine environment, costimulatory molecules expressed by the DC and type and load of presented antigen (Constant et al., 1995; Kuchroo et al., 1995; Shortman and Liu, 2002; Kaiko et al., 2007). Th1 cells, which secrete IFN-γ, are effective against intracellular pathogens, whereas Th2 cells secreting IL-4, IL-5, IL-10 and IL-13 target helminths and other extracellular parasites by activating B cells, eosinophils and mast cells (Kaiko et al., 2007). The recently described lineage, namely the Th17 cells, secretes IL-17, IL-6, IL-22 and TNF-α and has been shown to play an important role in inflammatory processes and activation of neutrophils (Harrington et al., 2005; Park et al., 2005; Langrish et al., 2005). The distinct Th lineages, and cytokines they produce, are antagonistic to each other; thus, usually, due to negative-feedback loops one lineage is dominant in response to one specific pathogen (Harrington et al., 2005; Kaiko et al., 2007). During maturation, DCs develop regulatory-, Th1-, Th2-, or Th17-inducing phenotype. Such matured DCs selectively produce cytokines, express coreceptors and several other polarizing signals that promote the development of certain regulatory or effector T cell lineages. Generally, Th1 polarizing factors include expression of IL-12, IL-18, IL-27, interferons and ICAM1 whereas IL-4, IL-6, IL-11, CD2 and histamine are ranked among Th2 polarizing factors (Farrar et al., 2002; Kidd, 2003; Kaiko et al., 2007; Dienz and Rincon, 2009). Th17 cells are presumed to develop from naïve precursors in the presence of IL-6 and TGF-β. Additionally, IL-23 is believed to be crucial in Th17 activation and survival (Harrington et al., 2005; Mangan et al., 2006).

Sitting at the boundary-line between innate and adaptive immunity, immature DCs are able to notice the nature of invading pathogens directly by recognizing their specific set of pathogen-associated molecular patterns (PAMPs) using evolutionarily highly conserved pattern-recognition receptors, including Toll-like receptors (TLRs) and lectins (Thoma-Uszynski et al., 2001; Janeway and Medzhitov, 2002; Underhill and Ozinsky, 2002; van Vliet et al., 2007). At the present time, 13 different TLRs have been described in mammals (Uematsu and Akira, 2007). Rapid virus recognition involves intracellular TLR3, TLR7, TLR8 and TLR9, where, generally, TLR3 recognizes double-stranded RNA (dsRNA), TLR7 and TLR8 recognize single-stranded RNA (ssRNA) and TLR9 is triggered by viral and bacterial DNA (Kawai and Akira, 2007; Kumagai et al., 2008). Additionally, surface

TLR2 and TLR4 have been shown to sense, besides a variety of bacterial and fungal PAMPs, envelope proteins of certain viruses including measles virus (MV) or human cytomegalovirus (HCMV) (Kawai and Akira, 2006).

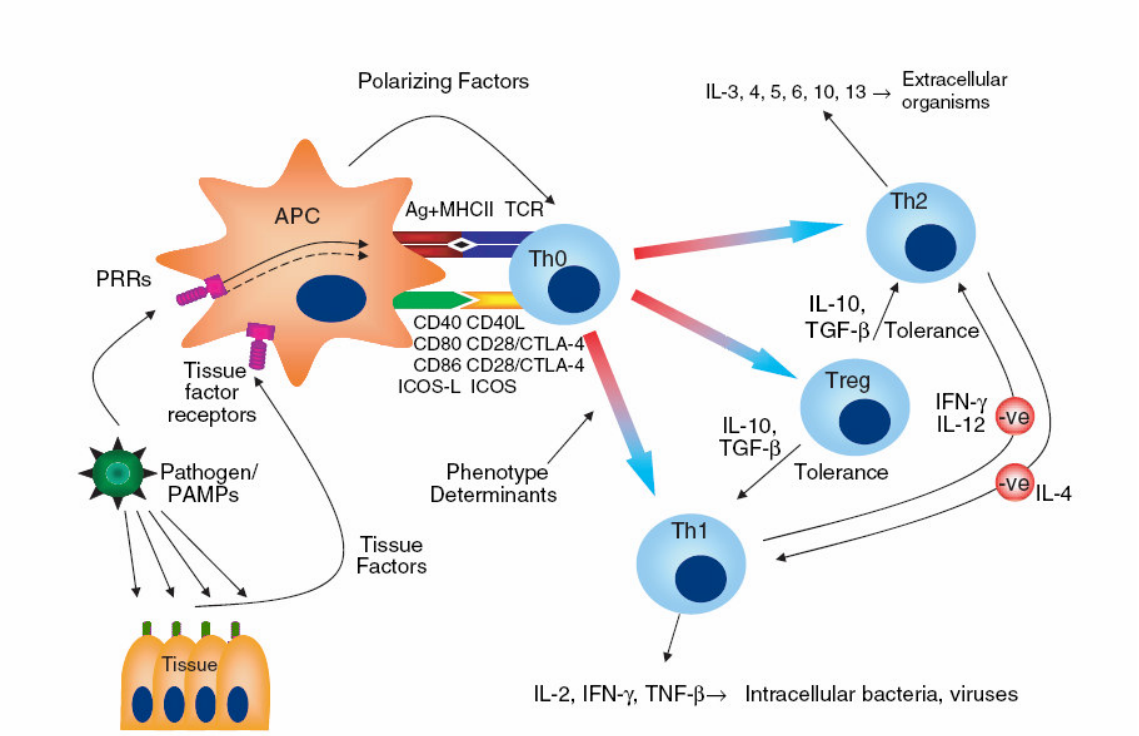

Fig. 1: Initiation of the adaptive immune response and determination of the T cell phenotype. Modified from Kaiko et al. (2007).

On the one hand, DCs are the key players in the induction of protective immunity to viral infections. On the other hand, DCs can also be exploited by viruses to circumvent the host immune response, induce immune suppression, or eventually even be employed as latent virus reservoirs (Pope et al., 1995; Grosjean et al., 1997; Fugier-Vivier et al., 1997; Kaiserlian and Dubois, 2001).

Some viruses, such as MV or HIV, use DCs as ferries to secondary lymphatic organs, where they can switch to T cells after a formation of an infectious synapse in DC/T cell conjugates (McDonald et al., 2003; Pohl et al., 2007). Many viruses can inhibit DC maturation by interference with antigen processing or downmodulation of MHC molecules, induce apoptosis (Engelmayer et al., 1999; Servet-Delprat et al., 2000; Palmer et al., 2005), or affect DC trafficking often by impairment of chemokine receptor switching (Moutaftsi et al., 2004; Prechtel et al., 2005). Most of the viruses studied in connection

with immune evasion have been shown to diminish the ability of DCs to stimulate naïve T cells. As mentioned above, such decreased capacity of efficient T cell priming can be due to the reduced expression of MHC molecules on DC surface. Similarly, expression of costimulatory molecules (including B7-1 and B7-2), which are known to amplify T cell receptor signalling, can be also targeted by viruses (Engelmayer et al., 1999; Servet-Delprat et al., 2000). In addition, infected DCs may directly provide inhibitory stimuli to T cells, for instance via upregulation of inhibitory molecules of the B7 family, such as B7-H1, which has been shown to interact with PD-1 (programmed death 1) molecule on T cells. Indeed, functionally impaired virus specific $CD8^+$ T cells, whose responses could be restored by blockage of PD-1/B7-H1 inhibitory pathway, have been recently observed in chronic viral infections (Barber et al., 2006). HCMV-infected DCs have been also shown to shed CD83 molecules. Such soluble CD83 have been reported to prevent efficient formation of DC/T cell conjugates and thus to inhibit T cell activation and proliferation (Lechmann et al., 2001; Sénéchal et al., 2004). Furthermore, the first pathogen noted to cause immunosupression, namely the MV, has been shown to inhibit T cell activation and proliferation directly through viral glycoproteins previously designated as T cell proliferation inhibiting factors (Schneider-Schaulies and Dittmer, 2006).

Taken together, DCs are functionally flexible cells that suit their response to signals they get from the environment including cytokines, chemokines, nature of the invading pathogen, and T cell proximity. Nevertheless, viruses, as well as other pathogens, have evolved mechanisms to avoid their recognition by the immune system via modification of the DC maturation programme, efficiency of migration, antigen presentation capacity and the outcome of DC/T cell interactions. It stands to reason that the interaction between DCs and viruses is a tricky game orchestrated by a wide spectrum of additional variables and a deeper understanding of these relationships will be crucial for DC-based immunotherapy especially for chronic autoimmune diseases or cancer.

2. Objectives

The general aim of the thesis is to describe the main effects of the tick saliva exerted on the initiation of adaptive immune responses and the implications of these effects on TBEV-dendritic cell interactions. Particular objectives of the key papers are as follows:

- to evaluate the effects of tick saliva exerted on dendritic cell phenotype and function in vitro and in vivo

- to describe the impact of TBEV infection on dendritic cell biology and subsequently to evaluate possible alteration of virus-dendritic cell interactions due to the presence of *I. ricinus* tick saliva

3. Publications

- **Skallová A,** Iezzi G, Ampenberger F, Kopf M, Kopecký J. Tick saliva inhibits dendritic cell migration, maturation, and function while promoting development of Th2 responses. J Immunol 2008, 180:6186-6192.

- **Skallová A,** Cimburek Z, Iezzi G, Kopecký J. *Ixodes ricinus* tick saliva modulates tick-borne encephalitis virus infection of dendritic cells. Manuscript ready for submission to the Journal of Infectious Diseases.

- Růžek D, Salát J, Palus M, Gritsun TS, Gould EA, Dyková I, **Skallová** A, Jelínek J, Kopecký J, Grubhoffer L. $CD8^+$ T-cells mediate immunopathology in tick-borne encephalitis. Virology 2009, 384:1-6.

Skallová A, Iezzi G, Ampenberger F, Kopf M, Kopecký J. Tick saliva inhibits dendritic cell migration, maturation, and function while promoting development of Th2 responses. J Immunol 2008, 180:6186-6192.

Similarly to other blood-feeding arthropods, ticks have evolved immunosuppressive mechanisms enabling them to overcome the host immune system. Although the immunomodulatory effect of tick saliva on several cell populations of the immune system has been extensively studied, little is known about its impact on dendritic cells (DCs). We have examined the effect of *Ixodes ricinus* tick saliva on DC function in vitro and in vivo. Exposure of DCs to tick saliva in vitro resulted in impaired maturation, upon CD40 or TLR9, TLR3 and TLR7 ligation, as well as reduced Ag presentation capacity. Administration of tick saliva in vivo significantly inhibited maturation and early migration of DCs from inflamed skin to draining lymph nodes, and decreased the capacity of lymph node DCs to present soluble Ag to specific T cells. Moreover, saliva-exposed DCs failed to induce efficient Th1 and Th17 polarization and promoted development of Th2 responses. Our data reveal a complex inhibitory effect exerted by tick saliva on DC function. Given the role of DCs as the key instigators of adaptive immune responses, alteration of their function might represent a major mechanism of tick-mediated immune evasion.

Skallová A, Cimburek Z, Iezzi G, Kopecký J. *Ixodes ricinus* tick saliva modulates tick-borne encephalitis virus infection of dendritic cells. Manuscript submitted to the Journal of Infectious Diseases.

Dendritic cells (DCs) are the early targets of tick-borne encephalitis virus (TBEV), an important human pathogen, naturally delivered into host skin via a tick bite. To examine the effect of TBEV on DC phenotype and function, we have cultured DCs with two TBEV strains of different virulence in the presence or absence of *Ixodes ricinus* tick saliva. We have demonstrated that TBEV induced IFN-β, TNF-α and IL-6 production, enhanced apoptosis and induced maturation of infected, but not bystander cells. Besides that, virus-exposed DCs stimulated proliferation of syngeneic effector $CD4^+$ T cells, but fail to prime naïve allogeneic T cells. Addition of the tick saliva enhanced proportion of TBEV-infected cells, led to a decrease in IFN-β, TNF-α and IL-6 levels, and reduced virus-induced apoptosis. Our data indicate that the tick saliva can modulate TBEV induced alterations in DCs, thus probably involving the early infection process in the host.

Růžek D, Salát J, Palus M, Gritsun TS, Gould EA, Dyková I, **Skallová** A, Jelínek J, Kopecký J, Grubhoffer L. $CD8^+$ T-cells mediate immunopathology in tick-borne encephalitis. Virology 2009, 384:1-6.

Epidemics of tick-borne encephalitis involving thousands of humans occur annually in the forested regions of Europe and Asia. Despite the importance of this disease, the underlying basis for the development of encephalitis remains undefined. Here, we prove the key role of CD8+ T-cells in the immunopathology of tickborne encephalitis, as demonstrated by prolonged survival of SCID or CD8−/− mice, following infection, when compared with immunocompetent mice or mice with adoptively transferred CD8+ T-cells. The results imply that tick-borne encephalitis is an immunopathological disease and that the inflammatory reaction significantly contributes to the fatal outcome of the infection.

4. Summary and Conclusions

Despite the crucial role of dendritic cells in immune responses to viral and parasitic infections, the interactions between these professional APCs and tick-borne viruses have not been evaluated so far. Similarly, only a few studies have focused on the effect of tick salivary bioactive molecules on DC biology. We described complex modulatory effects of *Ixodes ricinu*s tick saliva on DC phenotype and function in vitro and in vivo. Additionally, we have shown that the presence of the tick saliva measurably involves outcome of DC infection by the TBEV.

In the first study, we have demonstrated that exposure of DCs to tick saliva in vitro led to impaired CD40L- or TLR ligand-induced maturation and to reduced antigen presentation capacity. Moreover, DCs incubated with the presence of saliva polarized naïve T cells preferentially to Th2 subset, whereas Th1 and Th17 polarization were significantly inhibited.

Consistently with the in vitro experiments, DCs isolated from skin draining lymph nodes of saliva-treated mice exhibited a less mature phenotype than DCs from control mice, as assessed by flow cytometry analysis of the MHC II and B7-2 expression on the cell surface. Impairment of maturation was obvious not only in skin DCs that migrated into the lymph nodes in response to experimentally induced inflammation, but also in resident DCs. However, these results may not clearly manifest a direct inhibitory effect of tick saliva on resident DC maturation, but might also be a consequence of immature DC recruitment from blood to lymph nodes in response to repeated saliva exposure.

Administration of the tick saliva in vivo further markedly diminished early migration of peripheral DCs from skin to draining lymph nodes under inflammatory conditions and decreased the capacity of lymph node DCs to present soluble antigens to specific T cells. It is supposed that migratory dermal DCs may play an important role in antigen delivery into draining lymph nodes, and, thus, in initiation of specific immune responses (Henri et al. 2007). Therefore, saliva-induced inhibition in DC migration could postpone the development of specific immunity to tick feeding. In addition, the poor antigen presentation capacity of saliva-exposed DCs might result in suboptimal activation of specific T cells. Indeed, we have observed significantly decreased $CD4^+$ T cell proliferation mediated by DCs exposed to the tick saliva in vitro as well as in vivo. Furthermore, T cells activated by DCs isolated from control mice differentiated toward Th1, whilst T cells primed by DCs from saliva-treated mice predominantly underwent Th2

polarization. Our findings support recent data published by Mejri and Brossard (2007), who suggest that murine spleen-derived DCs pulsed with *I. ricinus* tick saliva favour the Th2 differentiation of $CD4^+$ T cells. Nevertheless, the design of this experiment does not give a clear evidence of saliva-induced Th2 polarization, since BALB/c mice used in this study tend to produce high levels of IL-4 in response to various signals including *Leishmania* and *Mycobacterium* infections (Huygen et al., 1992; Sacks and Noben-Trauth, 2002). Therefore, the specificity of the observed effect should probably be judged more carefully in this case.

As mentioned in the chapter *Introduction*, development of certain effector T helper lineages is shaped by a wide range of factors including the prevailing cytokine environment and character of co-stimulatory molecules expressed on DC surfaces (Constant et al., 1995; Kuchroo et al., 1995; Shortman and Liu, 2002; Kaiko et al., 2007). In our study, the decreased ability of tick saliva-exposed DCs to prime Th1 cells could be related to the inhibition of their capacity to produce cytokines after stimulation, as recently reported for BMDCs cells (Sá-Nunes et al., 2007). Consistent with these observations, we have also found reduced IL-6 and IL-12p70 concentrations in culture supernatants of CpG-activated spleen DCs in the presence of the saliva. Additionally, it has been reported that decreased expression of MHC and costimulatory molecules on DC surface led to preferential expansion of Th2 effector cells (Constant et al., 1995; Kuchroo et al., 1995). Thus, impairment of phenotypic and functional maturation of DCs seems to be a crucial immunomodulatory effect of the tick saliva in consequence of its following impact on the fine-tuning of the specific immune responses.

Differentiation into a Th2 subset is supposed to be advantageous for the tick. Indeed, an acute Th1-mediated inflammation of host skin may enhance the risk of parasite removal during grooming (Alexander, 1986) and development of the Th1-associated delayed-type hypersensitivity reaction at the tick bite-site is known to impact tick viability and fitness (Mosmann and Coffman, 1989; Ferreira et al., 2003). Additionally, it has been shown that polarization of the immune response toward the Th2 subset creates convenient conditions for the tick-transmitted spirochete *Borrelia burgdorferi* (Zeidner et al., 1997). These data suggest that tick saliva-induced alteration of DC function may be favourable not only for the tick, but also for certain tick-borne pathogens.

Up to now, two molecules contained in the saliva of the tick *I. scapularis* have been found to impair cytokine production by DCs as well as their capacity to induce T cell proliferation, namely prostaglandin E2 (Sá-Nunes et al. 2007) and Salp15 (Hovius et al.,

2008b). Whether some of these molecules could influence DC-mediated T cell polarization has not been evaluated so far; nevertheless, neither PGE2 nor Salp15 has been shown to inhibit expression of MHC II and co-stimulatory molecules on the surface of DCs. The effect of Salp15 on DC migration also remains to be addressed, however, PGE2 has been reported to induce DC migration rather than diminish it (Legler et al., 2006). Thus, we suppose that some additional molecules might be further involved in the complex inhibition of DC biology observed in our experiments.

Our following study was focused on the interactions among dendritic cells, tick saliva and TBEV. As mentioned above, TBEV is naturally delivered into the host skin during the blood sucking of an infected tick. At the infestation site, the virus has been shown to replicate in skin migratory DCs, as well as macrophages and neutrophils recruited from the blood (Labuda et al., 1996). However, the outcome of interactions between TBEV and DCs has not been evaluated so far. Considering that TBEV usually encounters the host together with bioactive tick salivary molecules, we decided to study not only the effects of TBEV infection on DC biology, but also possible alterations of these effects due to the presence of *I. ricinus* tick saliva.

We have demonstrated that infection by both of the TBEV strains used, namely the highly virulent strain Hypr and less virulent strain Neudoerfl, led to DC maturation, IFN-β, TNF-α and IL-6 production, and to decreased viability of virus-exposed cells in comparison with mock-infected cells. Despite the observed DC maturation upon virus exposure, infection with the highly virulent strain Hypr resulted in decreased antigen presentation capacity of DCs in mixed lymphocyte reaction (MLR). However, Hypr-exposed DCs were still able to prime effector syngeneic $CD4^+$ T cells.

In addition, we have shown that the proportion of TBEV-infected cells is enhanced in the presence of the tick saliva. The mechanism of this enhancement remains unknown; however, inflammatory cytokines, such as TNF-α, have been shown to down-regulate expression of DC-SIGN, a C-type lectin specific for human DCs (Relloso et al., 2002), which has been reported to play a critical role in binding of various pathogens including Dengue virus (Tassaneetrithep et al., 2003), HIV (Geijtenbeek et al., 2000), HCMV (Halary et al., 2002), Ebola virus (Baribaud et al., 2002) and *Leishmania* amastigotes (Colmenares et al., 2002) to host cells. We suggest that tick saliva-induced reduction in TNF-α production might lead to an increased expression of DC-SIGN or an equivalent molecule by mouse DCs, thus probably render DCs more permissive to flaviviral infection.

The exposure of the tick saliva also led to decreased apoptosis and to a reduction in TNF-α and IL-6 levels in culture supernatants, but had only a minimal effect on the expression of MHC class II and co-stimulatory molecules on surface of DCs. Despite a weak influence on the maturation markers, the tick saliva partly restored the Hypr-induced impairment of DC capacity to stimulate naïve $CD4^+$ T cells in the MLR. We suppose that this effect might occur probably due to the reduced apoptosis in saliva-treated cultures.

To our knowledge this study described for the first time the outcome of interactions between DCs and TBEV. Moreover, our findings indicate that TBEV induced alterations in mouse DC phenotype and function can be significantly modulated by the presence of the *I. ricinus* tick saliva. The tick saliva enhanced proportion of infected cells and markedly reduced production of proinflammatory cytokines, such as TNF- α. Together with IL-1, TNF- α is known to promote DC migration from the skin into regional lymph nodes (Roake et al., 1995; Cumberbatch et al., 1997). Indeed, in our first study summarized above, we have shown that the tick saliva impaired the early migration of skin DCs from inflamed skin. Considering that the saliva also reduced TBEV-mediated DC apoptosis, this data may suggest that in the presence of the tick saliva, infected DCs might stay in the infested skin for a prolonged time as a further source of the virus ready to be taken up by its tick vector. Thus, the tick saliva could in this way enhance the possibility of TBEV transmission among infected and uninfected ticks during their co-feeding on the same host. Nevertheless, whether or not the interactions among DCs, TBEV and the saliva of its tick vector could significantly involve the initial stage of virus replication or virus accessibility for feeding ticks in vivo needs further examination.

Taken together, we suggest that a substantial part of the tick saliva-mediated effects on host adaptive immune responses might have their origin in modulation of dendritic cell function. Indeed, DCs are known to initiate and shape adaptive immunity by priming and polarizing naïve T cells into effector or regulatory subtypes; thus, alteration of their phenotype and function may be enough for a significant shift of the final immune response in a direction that is favourable for the tick. Additionally, tick-friendly environments may also be advantageous for tick-borne pathogens, since DCs with impaired antigen presentation capacity due to the activities of the tick saliva fail to present not only salivary antigens, but also antigens derived from hitch-hikers, such as TBEV, delivered into the bargain to the host during tick feeding. In this regard tick saliva might help tick-borne

pathogens to evade the host immune system and possibly facilitate their transmission to new, uninfected ticks.

5. References

- Albrecht P. Pathogenesis of neurotropic arbovirus infections. *Curr Top Microbiol Immunol.* 1968, 43:44-91.

- Alekseev AN, Chunikhin SP, Rukhkian MIa, Stefutkina LF. The possible role of the salivary gland substrate in ixodid ticks as an adjuvant enhancing arbovirus transmission. *Med Parazitol*. 1991, 1:28-31.

- Alexander JO. The physiology of itch. *Parasitol Today* 1986, 2:345–351.

- Aljamali MN, Bior AD, Sauer JR, Essenberg RC. RNA interference in ticks: a study using histamine binding protein dsRNA in the female tick *Amblyomma americanum*. *Insect Mol Biol.* 2003, 12:299-305.

- Allen JR, Khalil HM, Wikel SK. Langerhans cells trap tick salivary gland antigens in tick-resistant guinea pigs. *J Immunol.* 1979, 122:563-565.

- Anguita J, Ramamoorthi N, Hovius JW, Das S, Thomas V, Persinski R, Conze D, Askenase PW, Rincón M, Kantor FS, Fikrig E. Salp15, an *Ixodes scapularis* salivary protein, inhibits CD4(+) T cell activation. *Immunity*. 2002, 16:849-59.

- Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006, 439:682-7.

- Baribaud F, Pöhlmann S, Leslie G, Mortari F, Doms RW. Quantitative expression and virus transmission analysis of DC-SIGN on monocyte-derived dendritic cells. *J Virol.* 2002, 76:9135-42.

- Basha G, Lizée G, Reinicke AT, Seipp RP, Omilusik KD, Jefferies WA. MHC class I endosomal and lysosomal trafficking coincides with exogenous antigen loading in dendritic cells. *PLoS ONE*. 2008, 3:e3247.

- Beaufays J, Adam B, Menten-Dedoyart C, Fievez L, Grosjean A, Decrem Y, Prévôt PP, Santini S, Brasseur R, Brossard M, Vanhaeverbeek M, Bureau F, Heinen E, Lins L, Vanhamme L, Godfroid E. Ir-LBP, an *Ixodes ricinus* tick salivary LTB4-binding lipocalin, interferes with host neutrophil function. *PLoS ONE*. 2008, 3:e3987.

- Belkaid Y, Kamhawi S, Modi G, Valenzuela J, Noben-Trauth N, Rowton E, Ribeiro J, Sacks DL. Development of a natural model of cutaneous leishmaniasis: powerful effects of vector saliva and saliva preexposure on the long-term outcome of *Leishmania major* infection in the mouse ear dermis. *J Exp Med*. 1998, 188:1941-53.

- Benditt EP, Bader S, Lam KB: Studies of the mechanism of acute vascular reactions to injury. I. The relationship of mast cells and histamine to the production of edema by ovomucoid in rats. *AMA Arch Pathol* 1955, 60:104-115.

- Bennouna S, Bliss SK, Curiel TJ, Denkers EY. Cross-talk in the innate immune system: neutrophils instruct recruitment and activation of dendritic cells during microbial infection. *J Immunol*. 2003, 171:6052-8.

- Bergman DK, Ramachandra RN, Wikel SK. Characterization of an immunosuppressant protein from *Dermacentor andersoni* (Acari: Ixodidae) salivary glands. *J Med Entomol.* 1998, 35:505-9.

- Bergman DK, Palmer MJ, Caimano MJ, Radolf JD, Wikel SK. Isolation and molecular cloning of a secreted immunosuppressant protein from *Dermacentor andersoni* salivary gland. *J Parasitol*. 2000, 86:516-25.

- Best SM, Morris KL, Shannon JG, Robertson SJ, Mitzel DN, Park GS, Boer E, Wolfinbarger JB, Bloom ME. Inhibition of interferon-stimulated JAK-STAT signaling by a tick-borne flavivirus and identification of NS5 as an interferon antagonist. *J Virol.* 2005, 79:12828-39.

- Bowessidjaou J, Brossard M, Aeschlimann A. Effects and duration of resistance acquired by rabbits on feeding and egg laying in *Ixodes ricinus* L. *Experientia.* 1977, 33:528-30.

- Brossard M, Wikel SK. Tick immunobiology. *Parasitology*. 2004, 129:S161-76.

- Brown SJ, Barker RW, Askenase PW. Bovine resistance to *Amblyomma americanum* ticks: an acquired immune response characterized by cutaneous basophil infiltrates. *Vet Parasitol*. 1984, 16:147-65.

- Brown SJ. Immunology of acquired resistance to ticks. *Parasitol Today* 1985, 1:165-171.

- Cavassani KA, Aliberti JC, Dias AR, Silva JS, Ferreira BR. Tick saliva inhibits differentiation, maturation and function of murine bone-marrow-derived dendritic cells. *Immunology*. 2005, 114:235-45.

- Colmenares M, Puig-Kröger A, Pello OM, Corbí AL, Rivas L. Dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN, CD209), a C-type surface lectin in human DCs, is a receptor for *Leishmania* amastigotes. *J Biol Chem*. 2002, 277:36766-9.

- Constant S, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J Exp Med.* 1995, 182:1591-6.

- Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol Rev*. 2005, 207:145-57.

- Cumberbatch M, Dearman RJ, Kimber I. Langerhans cells require signals from both tumour necrosis factor-α and interleukin-1β for migration. Immunology 1997; 92: 388-95.

- Daix V, Schroeder H, Praet N, Georgin JP, Chiappino I, Gillet L, de Fays K, Decrem Y, Leboulle G, Godfroid E, Bollen A, Pastoret PP, Gern L, Sharp PM, Vanderplasschen A. *Ixodes* ticks belonging to the *Ixodes ricinus* complex encode a family of anticomplement proteins. *Insect Mol Biol*. 2007, 16:155-66.

- denHollander N, Allen JR. *Dermacentor variabilis:* acquired resistance to ticks in BALB/c mice. *Exp Parasitol.* 1985, 59:118-29.
- Déruaz M, Frauenschuh A, Alessandri AL, Dias JM, Coelho FM, Russo RC, Ferreira BR, Graham GJ, Shaw JP, Wells TN, Teixeira MM, Power CA, Proudfoot AE. Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. *J Exp Med*. 2008, 205:2019-31.

- Dienz O, Rincon M. The effects of IL-6 on CD4 T cell responses. *Clin Immunol.* 2009, 130:27-33.

- Dizij A, Kurtenbach K. *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* L., the main European vector of *Borrelia burgdorferi*. *Parasite Immunol.* 1995, 17:177-83.

- Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. *Clin Infect Dis.* 1999, 28:882-90.

- Duniewicz M, Mertenova J, Moravcova E, et al. Central European tick-borne encephalitis from 1969 to 1972 in Central Bohemia. *Infection* 1975, 3**:**223-28.

- Ecker M, Allison SL, Meixner T, Heinz FX. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. *J Gen Virol.* 1999, 80:179-85.

- Engelmayer J, Larsson M, Subklewe M, Chahroudi A, Cox WI, Steinman RM, Bhardwaj N. Vaccinia virus inhibits the maturation of human dendritic cells: a novel mechanism of immune evasion. *J Immunol*. 1999, 163:6762-8.

- Farrar JD, Asnagli H, Murphy KM. T helper subset development: roles of instruction, selection, and transcription. *J Clin Invest* 2002, 109:431–5.

- Ferreira BR, Silva JS. Saliva of *Rhipicephalus sanguineus* tick impairs T cell proliferation and IFN-gamma-induced macrophage microbicidal activity. *Vet Immunol Immunopathol.* 1998, 64:279-93.

- Ferreira BR, Silva JS. Successive tick infestations selectively promote a T-helper 2 cytokine profile in mice. *Immunology.* 1999, 96:434-9.

- Ferreira BR, Szabó MJ, Cavassani KA, Bechara GH, Silva JS. Antigens from *Rhipicephalus sanguineus* ticks elicit potent cell-mediated immune responses in resistant but not in susceptible animals. *Vet Parasitol.* 2003, 115:35-48.

- Fokina GI, Malenko GV, Levina LS, Koreshkova GV, Rzhakhova OE, Mamonenko LL, Pogodina V, Frolova MP. Persistence of tick-borne encephalitis virus in monkeys. V. Virus localization after subcutaneous inoculation. *Acta Virol.* 1982, 26:369-375.

- Frauenschuh A, Power CA, Déruaz M, Ferreira BR, Silva JS, Teixeira MM, Dias JM, Martin T, Wells TN, Proudfoot AE. Molecular cloning and characterization of a highly selective chemokine-binding protein from the tick *Rhipicephalus sanguineus. J Biol Chem.* 2007, 282:27250-8.

- Fuchsberger N, Kita M, Hajnicka V, Imanishi J, Labuda M, Nuttall PA. Ixodid tick salivary gland extracts inhibit production of lipopolysaccharide-induced mRNA of several different human cytokines. *Exp Appl Acarol.* 1995, 19:671-6.

- Fugier-Vivier I, Servet-Delprat C, Rivailler P, Rissoan MC, Liu YJ, Rabourdin-Combe C. Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. *J Exp Med.* 1997, 186:813-23.

- Ganapamo F, Rutti B, Brossard M. In vitro production of interleukin-4 and interferon-gamma by lymph node cells from BALB/c mice infested with nymphal Ixodes ricinus ticks. *Immunology.* 1995, 85:120-4.

- Garg R, Juncadella IJ, Ramamoorthi N, Ashish, Ananthanarayanan SK, Thomas V, Rincón M, Krueger JK, Fikrig E, Yengo CM, Anguita J. Cutting edge: CD4 is the receptor for the tick saliva immunosuppressor, Salp15. *J Immunol*. 2006, 177:6579-83.

- Gasque P. Complement: a unique innate immune sensor for danger signals. *Mol Immunol.* 2004, 41:1089-98.

- Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen IL, Nottet HS, KewalRamani VN, Littman DR, Figdor CG, van Kooyk Y. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell.* 2000, 100:587-97.

- Gillespie RD, Mbow ML, Titus RG. The immunomodulatory factors of bloodfeeding arthropod saliva. *Parasite Immunol.* 2000, 22:319-31.

- Gillespie RD, Dolan MC, Piesman J, Titus RG. Identification of an IL-2 binding protein in the saliva of the Lyme disease vector tick, *Ixodes scapularis*. *J Immunol.* 2001, 166:4319-26.

- Gould EA, Buckley A, Barrett ADT, Cammack N. Neutralizing (54-kilodalton) and non-neutralizing (54-kilodalton and 48-kilodalton) monoclonal antibodies against structural and non-structural Yellow fever virus proteins confer immunity in mice. *J. Gen. Virol.* 1986, 67:591-595.

- Grešíková M, Sekeyová M, Stupalová S, Nečas S. Sheep milk-borne epidemic of tick-borne encephalitis in Slovakia. *Intervirology* 1975, 5:57-61.

- Grešíková M, Kaluzová M. Biology of tick-borne encephalitis virus. *Acta virol.* 1997, 41:115-124.

- Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. *Antivir Res.* 2003, 57:129-146.

- Grosjean I, Caux C, Bella C, Berger I, Wild F, Banchereau J, Kaiserlian D. Measles virus infects human dendritic cells and blocks their allostimulatory properties for CD4+ T cells. *J Exp Med*. 1997, 186:801-12.

- Gumperz JE, Brenner MB. CD1-specific T cells in microbial immunity. *Curr Opin Immunol.* 2001, 13:471-8.

- Gwakisa P, Yoshihara K, Long To T, Gotoh H, Amano F, Momotani E. Salivary gland extract of Rhipicephalus appendiculatus ticks inhibits in vitro transcription and secretion of cytokines and production of nitric oxide by LPS-stimulated JA-4 cells. *Vet Parasitol*. 2001, 99:53-61.

- Hajnická V, Kocáková P, Sláviková M, Slovák M, Gasperík J, Fuchsberger N, Nuttall PA. Anti-interleukin-8 activity of tick salivary gland extracts. *Parasite Immunol.* 2001, 23:483-9.

- Hajnická V, Vancová I, Kocáková P, Slovák M, Gasperík J, Sláviková M, Hails RS, Labuda M, Nuttall PA. Manipulation of host cytokine network by ticks: a potential gateway for pathogen transmission. *Parasitology.* 2005, 130:333-42.

- Halary F, Amara A, Lortat-Jacob H, Messerle M, Delaunay T, Houlès C, Fieschi F, Arenzana-Seisdedos F, Moreau JF, Déchanet-Merville J. Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. *Immunity.* 2002, 17:653-64.

- Hannier S, Liversidge J, Sternberg JM, Bowman AS. *Ixodes ricinus* tick salivary gland extract inhibits IL-10 secretion and CD69 expression by mitogen-stimulated murine splenocytes and induces hyporesponsiveness in B lymphocytes. *Parasite Immunol*. 2003, 25:27-37.

- Hannier S, Liversidge J, Sternberg JM, Bowman AS. Characterization of the B-cell inhibitory protein factor in *Ixodes ricinus* tick saliva: a potential role in enhanced *Borrelia burgdoferi* transmission. *Immunology.* 2004, 113:401-8.

- Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol*. 2005, 6:1123-32.

- Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol*. 2001, 19:47-64.

- Heinz FX. Epitope mapping of flavivirus glycoproteins. *Adv Virus Res.* 1986, 31:103-168.

- Henri S, Siret C, Machy P, Kissenpfennig A, Malissen B, Leserman L. Mature DC from skin and skin-draining LN retain the ability to acquire and efficiently present targeted antigen. *Eur J Immunol.* 2007, 37:1184–1193.

- Herzig R, Patt CM, Prokes T. An uncommon severe clinical course of European tick-borne encephalitis. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2002, 146:63-67.

- Hovius JW, Schuijt TJ, de Groot KA, Roelofs JJ, Oei GA, Marquart JA, de Beer R, van 't Veer C, van der Poll T, Ramamoorthi N, Fikrig E, van Dam AP. Preferential protection of *Borrelia burgdorferi* sensu stricto by a Salp15 homologue in *Ixodes ricinus* saliva. *J Infect Dis*. 2008a, 198:1189-97.

- Hovius JW, de Jong MA, den Dunnen J, Litjens M, Fikrig E, van der Poll T, Gringhuis SI, Geijtenbeek TB. Salp15 binding to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization. *PLoS Pathog*. 2008b, 4:e31.

- Huygen K, Abramowicz D, Vandenbussche P, Jacobs F, De Bruyn J, Kentos A, Drowart A, Van Vooren JP, Goldman M. Spleen cell cytokine secretion in *Mycobacterium bovis* BCG-infected mice. *Infect Immun*. 1992, 60:2880-6.

- Jacobs SC, Stephenson JR, Wilkinson GW. High-level expression of the tick-borne encephalitis virus NS1 protein by using an adenovirus-based vector: protection elicited in a murine model. *J Virol* 1992, 66:2086-2095.

- Janeway CA Jr, Medzhitov R. Innate immune recognition. *Annu Rev Immunol*. 2002, 20:197-216.

- Jones LD, Davies CR, Steele GM, Nuttall PA. A novel mode of arbovirus transmission involving a nonviremic host. Science. 1987, 237:775-7.

- Juncadella IJ, Garg R, Ananthnarayanan SK, Yengo CM, Anguita J. T-cell signaling pathways inhibited by the tick saliva immunosuppressor, Salp15. *FEMS Immunol Med Microbiol.* 2007, 49:433-8.

- Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology.* 2008, 123:326-38.

- Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994–98: a prospective study of 656 patients. *Brain* 1999, 122**:**2067-78.

- Kaiserlian D, Dubois B. Dendritic cells and viral immunity: friends or foes? *Semin Immunol*. 2001**,** 13:303-10.

- Kawai T, Akira S. Innate immune recognition of viral infection. *Nat Immunol*. 2006, 7:131-7.

- Kawai T, Akira S. Antiviral signaling through pattern recognition receptors. *J Biochem.* 2007, 141:137-45.

- Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev*. 2003, 8:223-46.

- Kopecký J, Kuthejlová M. Suppressive effect of *Ixodes ricinus* salivary gland extract on mechanisms of natural immunity in vitro. *Parasite Immunol*. 1998, 20:169-74.

- Kopecký J, Kuthejlová M, Pechová J. Salivary gland extract from Ixodes ricinus ticks inhibits production of interferon-gamma by the upregulation of interleukin-10. *Parasite Immunol*. 1999, 21:351-6.

- Kotsyfakis M, Sá-Nunes A, Francischetti IM, Mather TN, Andersen JF, Ribeiro JM. Antiinflammatory and immunosuppressive activity of sialostatin L, a salivary cystatin from the tick Ixodes scapularis. *J Biol Chem.* 2006, 281:26298-307.

- Kovář L, Kopecký J, Říhová B. Salivary gland extract from *Ixodes ricinus* tick polarizes the cytokine profile toward Th2 and suppresses proliferation of T lymphocytes in human PBMC culture. *J Parasitol*. 2001, 87:1342-8.

- Kročová Z, Macela A, Hernychová L, Kroča M, Pechová J, Kopecký J. Tick salivary gland extract accelerates proliferation of *Francisella tularensis* in the host. *J Parasitol.* 2003, 89:14-20.

- Kubeš M, Fuchsberger N, Labuda M, Zuffová E, Nuttall PA. Salivary gland extracts of partially fed *Dermacentor reticulatus* ticks decrease natural killer cell activity in vitro. *Immunology*. 1994, 82:113-6.

- Kubeš M, Kocáková P, Slovák M, Sláviková M, Fuchsberger N, Nuttall PA. Heterogeneity in the effect of different ixodid tick species on human natural killer cell activity. *Parasite Immunol*. 2002, 24:23-8.

- Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell*. 1995, 80:707-18.

- Kumagai Y, Takeuchi O, Akira S. TLR9 as a key receptor for the recognition of DNA. *Adv Drug Deliv Rev.* 2008, 60:795-804.
- Kuthejlová M, Kopecký J, Stepánová G, Macela A. Tick salivary gland extract inhibits killing of *Borrelia afzelii* spirochetes by mouse macrophages. *Infect Immun*. 2001, 69:575-8.
- Kýčková K, Kopecký J. Effect of tick saliva on mechanisms of innate immune response against *Borrelia afzelii*. *J Med Entomol.* 2006, 43:1208-14.
- Labuda M, Nuttall PA, Kozuch O, Elecková E, Williams T, Zuffová E, Sabó A. Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature. *Experientia*. 1993a, 49:802-5.
- Labuda M, Jones LD, Williams T, Nuttall PA. Enhancement of tick-borne encephalitis virus transmission by tick salivary gland extracts. *Med Vet Entomol.* 1993b, 7:193-6.
- Labuda M, Austyn JM, Zuffova E, Kozuch O, Fuchsberger N, Lysy J, Nuttall PA. Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology* 1996, 219**:**357-366.
- Labuda M, Kozuch O, Zuffova E, Eleckova E, Hails RS, Nuttall PA. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology* 1997, 235**:**138-143.
- Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med.* 2005, 201:233-40.
- Leboulle G, Crippa M, Decrem Y, Mejri N, Brossard M, Bollen A, Godfroid E. Characterization of a novel salivary immunosuppressive protein from *Ixodes ricinus* ticks. *J Biol Chem*. 2002, 277:10083-9.
- Lechmann M, Krooshoop DJ, Dudziak D, Kremmer E, Kuhnt C, Figdor CG, Schuler G, Steinkasserer A. The extracellular domain of CD83 inhibits dendritic cell-mediated T cell stimulation and binds to a ligand on dendritic cells. *J Exp Med*. 2001, 194:1813-21.
- Legler DF, Krause P, Scandella E, Singer E, Groettrup M. Prostaglandin E2 is generally required for human dendritic cell migration and exerts its effect via EP2 and EP4 receptors. *J Immunol*. 2006, 176:966-73.
- Lindenbach BD, Rice CM. Molecular biology of flaviviruses. *Adv Virus Res.* 2003, 59:23-61.
- Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*. 2006, 441:231-4.
- Masurier C, Salomon B, Guettari N, Pioche C, Lachapelle F, Guigon M, Klatzmann D. Dendritic cells route human immunodeficiency virus to lymph nodes after vaginal or intravenous administration to mice. *J Virol.* 1998, 72:7822-9.
- Matzinger P. The danger model: a renewed sense of self. *Science*. 2002, 296:301-5.

- Mbow ML, Rutti B, Brossard M. IFN-gamma IL-2, and IL-4 mRNA expression in the skin and draining lymph nodes of BALB/c mice repeatedly infested with nymphal *Ixodes ricinus* ticks. *Cell Immunol.* 1994, 156:254-61.

- McDonald D, Wu L, Bohks SM, KewalRamani VN, Unutmaz D, Hope TJ. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science*. 2003, 300:1295-7.

- Mejri N, Franscini N, Rutti B, Brossard M. Th2 polarization of the immune response of BALB/c mice to *Ixodes ricinus* instars, importance of several antigens in activation of specific Th2 subpopulations. *Parasite Immunol.* 2001, 23:61-9.

- Mejri N, Rutti B, Brossard M. Immunosuppressive effects of *Ixodes ricinus* tick saliva or salivary gland extracts on innate and acquired immune response of BALB/c mice. *Parasitol Res*. 2002, 88:192-7.

- Mejri N, Brossard M. Splenic dendritic cells pulsed with *Ixodes ricinus* tick saliva prime naive CD4+T to induce Th2 cell differentiation in vitro and in vivo. *Int Immunol.* 2007, 19:535-43.

- Mickiené A, Vene S, Golovljova I, Laiskonis A, Lindquist L, Plyusnin A, Lundkvist A. Tick-borne encephalitis virus in Lithuania. *Eur J Clin Microbiol Infect Dis.* 2001, 20:886-8.

- Montgomery RR, Lusitani D, De Boisfleury Chevance A, Malawista SE. Tick saliva reduces adherence and area of human neutrophils. *Infect Immun*. 2004, 72:2989-94.

- Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol.* 1989, 7:145-173.

- Moutaftsi M, Brennan P, Spector SA, Tabi Z. Impaired lymphoid chemokine-mediated migration due to a block on the chemokine receptor switch in human cytomegalovirus-infected dendritic cells. *J Virol*. 2004, 78:3046-54.

- Mulenga A, Macaluso KR, Simser JA, Azad AF. The American dog tick, *Dermacentor variabilis*, encodes a functional histamine release factor homolog. *Insect Biochem Mol Biol.* 2003, 33:911-9.

- Mulenga A, Azad AF. The molecular and biological analysis of ixodid ticks histamine release factors. *Exp Appl Acarol.* 2005, 37:215-29.

- Nazario S, Das S, de Silva AM, Deponte K, Marcantonio N, Anderson JF, Fish D, Fikrig E, Kantor FS. Prevention of *Borrelia burgdorferi* transmission in guinea pigs by tick immunity. *Am J Trop Med Hyg*. 1998, 58:780-5.

- Nithiuthai S, Allen JR. Langerhans cells present tick antigens to lymph node cells from tick-sensitized guinea-pigs. *Immunology*. 1985, 55:157-63.

- Nuttall PA, Jones LD. Non-viraemic tickborne virus transmission: mechanism and significance. In *Modern Acarology* (ed. Dusbabek, F., Bukva, V.) 1991, pp. 3–6. Prague and The Hague, Academia and SPB Academic Publishing.

- Nuttall PA, Labuda M. Tick-host interactions: saliva-activated transmission. *Parasitology*. 2004, 129:S177-89.
- Oliveira CJ, Cavassani KA, Moré DD, Garlet GP, Aliberti JC, Silva JS, Ferreira BR. Tick saliva inhibits the chemotactic function of MIP-1alpha and selectively impairs chemotaxis

of immature dendritic cells by down-regulating cell-surface CCR5. *Int J Parasitol*. 2008, 38:705-16.

- Osorio JE, Godsey MS, Defoliart GR, Yuill TM. La Crosse viremias in white-tailed deer and chipmunks exposed by injection or mosquito bite. *Am J Trop Med Hyg*. 1996, 54:338-42.

- Paesen GC, Adams PL, Harlos K, Nuttall PA, Stuart DI. Tick histamine-binding proteins: isolation, cloning, and three-dimensional structure. *Mol Cell.* 1999, 3:661-71.

- Palmer DR, Sun P, Celluzzi C, Bisbing J, Pang S, Sun W, Marovich MA, Burgess T. Differential effects of dengue virus on infected and bystander dendritic cells. *J Virol*. 2005, 79:2432-9.

- Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol*. 2005, 6:1133-41.

- Pechová J, Stěpánová G, Kovár L, Kopecký J. Tick salivary gland extract-activated transmission of *Borrelia afzelii* spirochaetes. *Folia Parasitol.* 2002, 49:153-9.

- Pogodina V, Frolova MP, Malenko GV, Fokina GI, Levina LS, Mamonenko LL, Koreshkova GV, Ralf NM. Persistence of tick-borne encephalitis virus in monkeys. I. Features of experimental infection. *Acta Virol.* 1981a, 25:337-343.

- Pogodina V, Levina LS, Fokina GI, Koreshkova GV, Malenko GV, Bochkova NG, Rzhakhova OE. Persistence of tick-borne encephalitis virus in monkeys. III. Phenotypes of the persisting virus. *Acta Virol.* 1981b, 25:352-360.

- Pohl C, Shishkova J, Schneider-Schaulies S. Viruses and dendritic cells: enemy mine. *Cell Microbiol.* 2007, 9:279-89.

- Pope M, Gezelter S, Gallo N, Hoffman L, Steinman RM. Low levels of HIV-1 infection in cutaneous dendritic cells promote extensive viral replication upon binding to memory CD4+ T cells. *J Exp Med*. 1995, 182:2045-56.

- Prechtel AT, Turza NM, Kobelt DJ, Eisemann JI, Coffin RS, McGrath Y, Hacker C, Ju X, Zenke M, Steinkasserer A. Infection of mature dendritic cells with herpes simplex virus type 1 dramatically reduces lymphoid chemokine-mediated migration. *J Gen Virol*. 2005, 86:1645-57.

- Ramachandra RN, Wikel SK. Modulation of host-immune responses by ticks (Acari: Ixodidae): effect of salivary gland extracts on host macrophages and lymphocyte cytokine production. *J Med Entomol*. 1992, 29:818-26.

- Ramamoorthi N, Narasimhan S, Pal U, Bao F, Yang XF, Fish D, Anguita J, Norgard MV, Kantor FS, Anderson JF, Koski RA, Fikrig E. The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature*. 2005, 436:573-7.

- Relloso M, Puig-Kröger A, Pello OM, Rodríguez-Fernández JL, de la Rosa G, Longo N, Navarro J, Muñoz-Fernández MA, Sánchez-Mateos P, Corbí AL. DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-beta, and anti-inflammatory agents. *J Immunol.* 2002, 168:2634-43.

- Ribeiro JM, Makoul GT, Levine J, Robinson DR, Spielman A. Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick, *Ixodes dammini*. *J Exp Med*. 1985, 161:332-44.

- Ribeiro JM, Spielman A. *Ixodes dammini*: salivary anaphylatoxin inactivating activity. *Exp Parasitol*. 1986, 62:292-7.

- Ribeiro JM. *Ixodes dammini*: salivary anti-complement activity. *Exp Parasitol.* 1987, 64:347-53.

- Ribeiro JM, Weis JJ, Telford SR 3rd. Saliva of the tick *Ixodes dammini* inhibits neutrophil function. *Exp Parasitol*. 1990, 70:382-8.

- Ribeiro JM, Mather TN. Ixodes scapularis: salivary kininase activity is a metallo dipeptidyl carboxypeptidase. *Exp Parasitol.* 1998, 89:213-21.

- Richerson HB, Dvorak HF, Leskowitz S. Cutaneous basophil hypersensitivity. I. A new look at the Jones-Mote reaction, general characteristics. *J Exp Med.* 1970, 132:546-57.

- Roake JA, Rao AS, Morris PJ, Larsen CP, Hankins DF, Austyn JM. Dendritic cell loss from nonlymphoid tissues after systemic administration of lipopolysaccharide, tumor necrosis factor, and interleukin 1. *J Exp Med***.** 1995, 181:2237-47.

- Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat Rev Immunol*. 2002, 2:845-58.

- Sangamnatdej S, Paesen GC, Slovak M, Nuttall PA. A high affinity serotonin- and histamine-binding lipocalin from tick saliva. *Insect Mol Biol.* 2002, 11:79-86.

- Sá-Nunes A, Bafica A, Lucas DA, Conrads TP, Veenstra TD, Andersen JF, Mather TN, Ribeiro JM, Francischetti IM. Prostaglandin E2 is a major inhibitor of dendritic cell maturation and function in Ixodes scapularis saliva. J Immunol. 2007, 179:1497-505.

- Schall TJ, Bacon K, Camp RD, Kaspari JW, Goeddel DV. Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. *J Exp Med.* 1993, 177:1821-6.

- Schneider H. Über epidemische akute „Meningitis serosa“. *Wien Klin Wochenschr.* 1931, 44:350-352.

- Schneider-Schaulies S, Dittmer U. Silencing T cells or T-cell silencing: concepts in virus-induced immunosuppression. *J Gen Virol*. 2006, 87:1423-38.

- Schoeler GB, Manweiler SA, Wikel SK. Ixodes scapularis: effects of repeated infestations with pathogen-free nymphs on macrophage and T lymphocyte cytokine responses of BALB/c and C3H/HeN mice. *Exp Parasitol.* 1999, 92:239-48.

- Schroeder H, Daix V, Gillet L, Renauld JC, Vanderplasschen A. The paralogous salivary anti-complement proteins IRAC I and IRAC II encoded by *Ixodes ricinus* ticks have broad and complementary inhibitory activities against the complement of different host species. *Microbes Infect.* 2007, 9:247-50.

- Sénéchal B, Boruchov AM, Reagan JL, Hart DN, Young JW. Infection of mature monocyte-derived dendritic cells with human cytomegalovirus inhibits stimulation of T-cell proliferation via the release of soluble CD83. *Blood*. 2004, 103:4207-15.

- Servet-Delprat C, Vidalain PO, Bausinger H, Manié S, Le Deist F, Azocar O, Hanau D, Fischer A, Rabourdin-Combe C. Measles virus induces abnormal differentiation of CD40 ligand-activated human dendritic cells. *J Immunol*. 2000, 164:1753-60.

- Shim WS, Oh U. Histamine-induced itch and its relationship with pain. *Mol Pain*. 2008, 4:29.

- Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol*. 2002, 2:151-61.

- Singh SK, Girschick HJ. Tick-host interactions and their immunological implications in tick-borne diseases. *Curr Sci.* 2003, 85: 1284-1298.

- Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol.* 1991, 9:271-96.

- Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med*. 2000, 191:411-6.

- Tassaneetrithep B, Burgess TH, Granelli-Piperno A, Trumpfheller C, Finke J, Sun W, Eller MA, Pattanapanyasat K, Sarasombath S, Birx DL, Steinman RM, Schlesinger S, Marovich MA. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J Exp Med*. 2003, 197:823-9.

- Ternovoi VA, Kurzhukov GP, Sokolov YV, Ivanov GY, Ivanisenko VA, Loktev AV, Ryder RW, Netesov SV, Loktev B. Tick-borne encephalitis with hemorrhagic syndrome, Novosibirsk Region, Russia, 1999. *Emerg Infect Dis.* 2003, 9:743-746.

- Thiel HJ, Collett MS, Gould EA, Heinz FX, Houghton M, Meyers G, Purcell RH, Rice C. 2005. Family Flaviviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. (Eds.), Virus Taxonomy: Classification and Nomenclature, Eighth Report of the International Committee on the Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, Boston, Heidelberg, London, New York, Oxford, pp. 981–998.

- Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, Barnes PF, Rollinghoff M, Bolcskei PL, Wagner M, Akira S, Norgard MV, Belisle JT, Godowski PJ, Bloom BR, Modlin RL. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science*. 2001, 291:1544-7.

- Timofeev AV, Ozherelkov SV, Pronin AV, Deeva AV, Karganova GG, Elbert LB, Stephenson JR. Immunological basis for protection in a murine model of tick-borne encephalitis by a recombinant adenovirus carrying the gene encoding the NS1 non-structural protein. *J Gen Virol.* 1998, 79:689-695.

- Titus RG, Bishop JV, Mejia JS. The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. *Parasite Immunol.* 2006, 28:131-41.

- Trager W. Acquired immunity to ticks. *J Parasitol.* 1939, 25:7-81.

- Turni C, Lee RP, Jackson LA. A comparison of the immunosuppressive effects of salivary gland extracts from two laboratory strains of *Boophilus microplus*. *Int J Parasitol.* 2004, 34:833-8.

- Tyson K, Elkins C, Patterson H, Fikrig E, de Silva A. Biochemical and functional characterization of Salp20, an *Ixodes scapularis* tick salivary protein that inhibits the complement pathway. *Insect Mol Biol.* 2007, 16:469-79.

- Uematsu S, Akira S. Toll-like receptors and Type I interferons. *J Biol Chem*. 2007, 282:15319-23.

- Underhill DM, Ozinsky A. Toll-like receptors: key mediators of microbe detection. *Curr Opin Immunol*. 2002, 14:103-10.

- Urioste S, Hall LR, Telford SR 3rd, Titus RG. Saliva of the Lyme disease vector, Ixodes dammini, blocks cell activation by a nonprostaglandin E2-dependent mechanism. *J Exp Med.* 1994, 180:1077-85.

- Valenzuela JG, Charlab R, Mather TN, Ribeiro JM. Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, *Ixodes scapularis*. J Biol Chem. 2000, 275:18717-23.

- Valenzuela JG, Francischetti IM, Pham VM, Garfield MK, Mather TN, Ribeiro JM. Exploring the sialome of the tick *Ixodes scapularis*. *J Exp Biol.* 2002, 205:2843-64.

- Valenzuela JG. Exploring tick saliva: from biochemistry to 'sialomes' and functional genomics. *Parasitology.* 2004, 129:S83-94.

- van Vliet SJ, den Dunnen J, Gringhuis SI, Geijtenbeek TB, van Kooyk Y. Innate signaling and regulation of Dendritic cell immunity. *Curr Opin Immunol*. 2007, 19:435-40.

- Vaughan JA, Scheller LF, Wirtz RA, Azad AF. Infectivity of *Plasmodium berghei* sporozoites delivered by intravenous inoculation versus mosquito bite: implications for sporozoite vaccine trials. *Infect Immun*. 1999, 67:4285-9.

- Votiakov I, Protas II, Zhdanov VM. Western Tick-Borne Encephalitis. Belarus, Minsk, 1978, 256 pp.

- Wang H, Nuttall PA. Excretion of host immunoglobulin in tick saliva and detection of IgG-binding proteins in tick haemolymph and salivary glands. *Parasitology*. 1994, 109:525-30.

- Wang H, Nuttall PA. Immunoglobulin-G binding proteins in the ixodid ticks, *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Ixodes hexagonus*. *Parasitology*. 1995, 111:161-5.

- Wang H, Nuttall PA. Immunoglobulin-binding proteins in ticks: new target for vaccine development against a blood-feeding parasite. *Cell Mol Life Sci*. 1999, 56:286-95.

- WHO Regional Office for Europe (WHO/EURO). The vector-borne human infections of Europe: Their distribution and burden on public health. Copenhagen, Denmark: WHO Regional Office for Europe (WHO/EURO), 2004, 37–53; http://www.euro.who. int/document/e82481.pdf

- Wikel SK. Host immunity to ticks. *Annu Rev Entomol.* 1996, 41:1-22.

- Wikel SK, Ramachandra RN, Bergman DK, Burkot TR, Piesman J. Infestation with pathogen-free nymphs of the tick *Ixodes scapularis* induces host resistance to transmission of *Borrelia burgdorferi* by ticks. *Infect Immun*. 1997, 65:335-8.

- Wikel SK. Tick modulation of host immunity: an important factor in pathogen transmission. *Int J Parasitol.* 1999, 29:851-9.

- Wilson NS, El-Sukkari D, Belz GT, Smith CM, Steptoe RJ, Heath WR, Shortman K, Villadangos JA. Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood.* 2003, 102:2187-94.

- Yewdell JW, Norbury CC, Bennink JR. Mechanisms of exogenous antigen presentation by MHC class I molecules in vitro and in vivo: implications for generating CD8+ T cell responses to infectious agents, tumors, transplants, and vaccines. *Adv Immunol.* 1999, 73:1-77.

- Zeidner NS, Mbow ML, Dolan MC, Baca E, Massung R, Piesman J. Effects of *Ixodes scapularis* and *Borrelia burgdorferi* on modulation of the host immune response: induction of a TH2 cytokine response in lyme disease susceptible (C3H/HeJ) mice but not in disease-resistant (BALB/c) mice. *Infect Immun.* 1997, 65:3100-3106.

- Zeidner NS, Schneider BS, Nuncio MS, Gern L, Piesman J. Coinoculation of *Borrelia* spp. with tick salivary gland lysate enhances spirochete load in mice and is tick species-specific. *J Parasitol*. 2002, 88:1276-8.

- Zilber LA. Vesennij (vesenne-letnij) epidemičeskij kleščevoj encefalit. *Archiv Biologicheskich Nauk* 1939, 56:2.