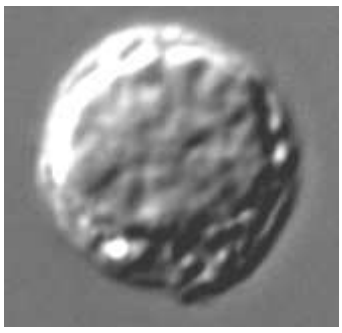
DAY OF IMMUNOLOGY 2012

‘TOWARD NEW FRONTIERS’

23 April 2012, PRAGUE

INSTITUTE OF MICROBIOLOGY, v.v.i.

ACADEMY OF SCIENCES OF THE CZECH REPUBLIC



To the 50th anniversary of the Institute of Microbiology foundation

Dedicated to the memory of Professor Jaroslav Šterzl

SPONSORS

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INVITATION

Dear colleagues,

The Institute of Microbiology of the Academy of Sciences of the Czech Republic, v.v.i., celebrates the 50th anniversary of foundation this year and in the frame of the celebrations a special Day of Immunology will take place on April 23, 2012.

This one-day symposium entitled "Day of Immunology 2012: Toward New Frontiers" will be dedicated to the memory of Professor Jaroslav Šterzl, MD., DrSc., the founder of modern Czechoslovak Immunology, who passed away on April 8, 2012, one month short of his 87th birthday.

He was a former head of the Department of Immunology and Director of the Institute of Microbiology of the Academy of Sciences of the Czech Republic (Czechoslovak at that time). His enormous contributions to immunology are recognized and remembered with gratitude and fondness.

For this celebrative symposium, we managed to collect several important speakers from abroad who will present the results of their recent research. Members of the Department of Immunology and Gnotobiology of the Institute of Microbiology will also participate with short presentations about their current studies.

All of you are warmly invited to participate to this intensive day of scientific discussions. Students and young scientists are welcome to get the possibility to exchange ideas with the panel of international experts.

This event was made possible by the cooperation of the Institute of Microbiology, the Czech Immunological Society, the Italian Institute of Culture in Prague, and our sponsors.

Looking forward to see you.

Cordially,

Luca Vannucci, MD., PhD., organizer

GENERAL INFORMATION

Venue

The symposium will take place in the

Institute of Microbiology, v.v.i.

Academy of Sciences of the Czech Republic

Vídeňská 1083, Prague 4-Krč,

142 20, Czech Republic

Transportation

Location is easily accessible by city bus No. 193, terminus stop "Ústavy Akademie věd". From the Metro line C, change at the "Budějovická" station. The bus can be taken at the bus stop "Poliklinika Budějovická", just in front of the new Municipal building of Prague 4.

The official language of this Symposium is English.

Web pages: www.biomed.cas.cz/DIMBU2012

We thank Dr. Ivan Janda for his precious work in preparing the web pages.

SPEAKERS

(in alphabetical order)

International panel

- Prof. Ron N. Apte, Ben-Gurion University of the Negev, Beer Sheva, Israel
- Prof. Paul V. Lehmann, Case Western Reserve University School of Medicine, and Cellular Technology Ltd., Cleveland, OH, USA
- Prof. Massimo Locati, Istituto Clinico Humanitas and University of Milan, Milan, Italy
- Prof. Jiri Mestecky, University of Alabama at Birmingham, Birmingham, AL, USA, and Institute of Microbiology, AS CR, Prague, Czech Republic
- Prof. Antonio Sica, Istituto Clinico Humanitas, Milan, and University of Eastern Piemont, Novara, Italy
- Prof. Pramod K. Srivastava, Center for Immunotherapy of Cancer and Infectious Diseases, University of Connecticut School of Medicine, Farmington, CT, USA
- Prof. Giorgio Trinchieri, NCI, Frederick, and NIH, Bethesda, MD, USA
- Prof. Viktor Umansky, German Cancer Research Center, Heidelberg, and University Hospital Mannheim, Germany

Institute of Microbiology

- Dr. Martin Bilej, DSc
- Dr. Marek Kovář, PhD
- Dr. Miloslav Kverka, PhD
- Dr. Marek Šinkora, PhD
- Dr. Luca Vannucci, PhD

PROGRAM

9:00 **Opening**

9:15	Prof. G. Trinchieri, USA	Innate resistance, inflammation, and carcinogenesis
9:50	Prof. R. Apte, Israel	Interleukin-1-mediated inflammation and the malignant process
10:25	Prof. M. Locati, Italy	Regulation of the chemokine system by atypical chemokine receptors

11:00 Coffee break

11:15	Prof. P. Lehmann, USA	PAMPS and alarmins in autoimmunity – lessons for cancer?
11:50	Prof. V. Umansky, Germany	Immunosuppression in tumor microenvironment induced by chronic inflammation
12:25	Dr. L. Vannucci, CZ	Tumor microenvironment as a target for selective immunotherapy
12:45	Dr. M. Kovář, CZ	Tuning the immunity up and down with complexes of IL-2 and anti-IL-2 mAb

13:05 Lunch

14:15	Prof. A. Sica, Italy	Macrophage plasticity and polarization in disease
14:50	Dr. M. Bilej, CZ	The role of lectin-saccharide interactions in the evolution of cytokines
15:10	Prof. P. Srivastava, USA	Peering deep into the heart of tumor-specificity

15:45 Coffee break

16:00	Prof. J. Mestecky, USA/CZ	IgA Nephropathy: autoimmune nature of the disease
16:35	Dr. M. Šinkora, CZ	The role of the ileal Peyer's patches and bone marrow in B cell development in swine
16:55	Dr. M. Kverka, CZ	Oral treatment with antigens from *Parabacteroides distasonis* protects mice from experimental colitis by oral tolerance induction

17:15 Panel discussion (Apte, Lehmann, Locati, Mestecky, Říhová, Sica, Srivastava, Trinchieri, Umansky, Vannucci)

17:45 Conclusion (Prof. B. Říhová, CZ)

17:50 Refreshment (until 18:45)

ABSTRACTS (authors in alphabetic order)

International speakers

Interleukin-1-mediated inflammation and the malignant process

Ron N. Apte[1], Peleg Rider[1], Idan Cohen[1], Yaron Carmi[1], Shahar Dotan[1], Moshe Elkabets[1], Irena Kaplanov[1], Rosalyn M. White[1], Charles A. Dinarello[2] and Elena Voronov[1]

[1]The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel, and [2]Department of Medicine, Division of Infectious Diseases, University of Colorado Denver, Aurora, CO, USA

Interleukin-1 (IL-1) is a pleiotropic pro-inflammatory and immunostimulatory cytokine with diverse effects in steady-state homeostasis and in disease. The IL-1 family consists of two agonistic proteins, namely IL-1α and IL-1β, and one antagonistic protein, the IL-1 receptor antagonist (IL-1Ra), which binds to IL-1 receptors without transmitting an activation signal. In their recombinant form, IL-1α and IL-1β bind to the same receptors and exert the same biological activities. However, in the physiological milieu, IL-α and IL-1β differ dramatically in the sub-cellular compartment in which they are found, which dictates distinct functions. Thus, IL-1α is mainly active as a cell-associated cytokine (cytosolic, nuclear and membrane-associated forms), while IL-1β is active only in its secreted mature form. As a ubiquitous mediator at tumor sites, IL-1 is produced by microenvironmental cellular elements as well as by the malignant cells. Cell-associated IL-1α translocates into the nucleus, especially upon stress, where it binds to chromatin in a dynamic manner. During necrosis of stressed cells, IL-1α is released into the microenvironment and initiates sterile inflammation. However, upon an apoptotic cell death, IL-1α binds avidly to chromatin and is not released from the cells and thus inflammation is not induced. For the propagation of the inflammatory response induced by IL-1α of damaged tissue origin, it synergizes with IL-1β secreted with bone marrow-derived myeloid cells, which results in an extended inflammatory response, characterized mainly by infiltrating mononuclear cells. Also, recombinant IL-1α is dominant in early recruitment of neutrophils, while recombinant IL-1β is more effective in recruiting mononuclear cells, further attesting differential roles to the IL-1 agonists. In the malignant process, extended inflammatory responses related to carcinogenesis, tumor cell invasiveness and tumor angiogenesis were mainly dependent on microenvironment-derived IL-1β which is mainly secreted by infiltrating myeloid cells, with some contribution to tumor cell-derived IL-1. In accordance, treatment of tumor-bearing mice with IL-1Ra or specific anti-IL-1 antibodies alleviated tumor burden. However, host-derived IL-1α, which is mostly cell-associated, is dominantly involved in the process of craving the immunogenic repertoire of the malignant cells in the process of immunoediting. Furthermore, IL-1 was shown to be a major cytokine, which affects the balance between the chronic wound-healing type of inflammation, which is involved in tumor progression, and activation of professional APCs in a limited inflammatory milieu, which leads to induction of anti-tumor cell immunity and reduction of the tumor burden. Better understanding the circumstances by which molecules of the IL-1 family affect the malignant process will broaden the use of IL-1 manipulation in tumor therapy.

PAMPS and alarmins in autoimmunity – lessons for cancer?

Paul V. Lehmann

Case Western Reserve University, Cleveland, OH, USA, and C.T.L.

Alarmins released during tissue injury have been recently linked to CD8 cells' ability to express effector functions. Cryogenic CNS injury, triggering local alarmin release, however, does not render the CNS susceptible to an attack by CNS antigen-reactive Th17 CD4 cells. In contrast, deposition of the PAMP, CpG, into the CNS does. Potentially pathogenic CD4 cells' ability to exert effector function in a target organ is therefore dependent on a local "third signal" which in EAE can be a PAMP, but not an alarmin. These findings may also apply for the autoimmune response against tumor antigens, and thus to tumor immunology.

Regulation of the chemokine system by atypical chemokine receptors

Massimo Locati

University of Milan and Istituto Clinico Humanitas IRCCS, Milan, Italy

Chemokines are a distinct large family of cytokines orchestrating leukocyte recruitment through activation of a dedicated panel of G protein-coupled receptors. A set of chemokine receptors indicated as "atypical", in that structurally unable to mediate direkt cell migration, are emerging as a major regulatory mechanism of chemokines biological activity. Atypical chemokine receptors are structurally and functionally heterogeneous, but they share the biological role of regulating signaling receptors' activity by clearance, transport, or presentation of their cognate ligands The atypical chemokine receptor D6 in particular is expressed on lymphatic vessels, binds and drives to degradation the majority of inflammatory CC chemokines, and by this chemokine scavenger function affects mobilization and trafficking of specific leukocyte subsets, including Ly6C^{high} monocytes and $\gamma\delta$ T cells, thus regulating inflammation and inflammation-driven tumor progression in different organs, including skin, liver, colon, lungs, and placenta.

IgA nephropathy: autoimmune nature of the disease

Jiri Mestecky

University of Alabama at Birmingham, Birmingham, AL, USA, and Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Studies of molecular and cellular interactions involved in the pathogenesis of the most common glomerulonephritis - IgA nephropathy - revealed the autoimmune nature of this disease. Altered glycan structures of the unique hinge region of the heavy chains in IgA1 molecules in patients with this disease lead to the exposure of antigenic determinants which are recognized by unique anti-glycan antibodies of IgG or IgA1 isotypes resulting in the formation of nephritogenic immune complexes in the circulation and their deposition in the

glomerular mesangium. Deposited immune complexes induce proliferation of resident mesangial cells, increased production of extracellular matrix proteins and cytokines, and ultimately the loss of glomerular function. Structural elucidation of the nature and biological activity of immune complexes should provide a rational basis for effective, immunologically-mediated interference with the formation of nephritogenic immune complexes as a disease-specific therapeutic approach.

Macrophage plasticity and polarization in disease

Antonio Sica

Istituto Clinico Humanitas IRCCS, Rozzano, and DiSCAFF, University of Piemonte Orientale, Novara, Italy

Diversity and plasticity are hallmarks of cells of the monocyte-macrophage lineage. In response to interferons, Toll-like receptor engagement or IL-4/IL-13 signaling, macrophages undergo M1 (classical) or M2 (alternative) activation, which represent extremes of a continuum in a universe of activation states. Progress has now been made in defining the signaling pathways, transcriptional networks and epigenetic mechanisms underlying M1-M2 or M2-like polarized activation. Functional skewing of mononuclear phagocytes occurs in vivo under physiological conditions (e.g. ontogenesis and pregnancy) and in pathology (allergic and chronic inflammation, tissue repair, infection and cancer). However, in selected preclinical and clinical conditions coexistence of cells in different activation states and unique or mixed phenotypes has been observed, a reflection of dynamic changes and complex tissue-derived signals. The identification of mechanisms and molecules associated with macrophage plasticity and polarized activation provides a basis for macrophage-centered diagnostic and therapeutic strategies.

Peering deep into the heart of tumor-specificity

Pramod K. Srivastava

University of Connecticut School of Medicine, Farmington, CT, USA

New sequencing technology permits us to peer deep into the tumor genomes and transcriptomes in a manner that was inconceivable even ten years ago. Our laboratory has characterized the transcriptomes of a small number of BALB/c mouse tumors, and compared the sequences of the transcripts to the normal genomic sequences. Thousand of non-synonymous mutations have been identified. A proportion of these have been predicted to form strong tumor-specific epitopes for each of the three MHCI alleles (K, D and L). These predictions have been tested experimentally in terms of the ability of these epitopes to elicit a functional CD8+ T cell response, and to modulate the kinetics of tumor growth. These results, which shed a powerful light on the identity of tumor-specific antigenic epitopes, shall be discussed. The application of this methodology to immunotherapy of human cancers shall also be discussed.

Innate resistance, inflammation, and carcinogenesis

Giorgio Trinchieri[1], Zsofia Gyulai[1], Rosalba Salcedo[1], Yava Jones[1], Lyudmila Lyakh[1], Robin Winkler Pickett[1], Loretta Smith[1], Ren Ming Dai[1], Anna Mason[1], Christophe Cataisson[2], Stuart Yuspa[2]

[1]Cancer and Inflammation Program, CCR, NCI, Frederick, MD, USA, and [2]Laboratory of Cell Biology and Genetics, CCR, NCI, Bethesda, MD, USA

The interaction of the inflammatory mediators and innate and immune effector cells with carcinogenesis and tumor progression is complicated and results in effects that either favor or impede tumor progression. The simple concept that early inflammation is necessary for carcinogenesis whereas inflammatory and immune response prevent tumor progression has been replaced by a more subtle understanding that the degree of inflammation and the type of inflammatory/immune response are responsible for tilting the balance between tumor progression and regression. Furthermore, it is becoming evident that the processes that the organisms use for resistance to infections are related to the mechanisms essential for tissue homeostasis and morphogenesis. In order to address these mechanisms in carcinogenesis, we studied the role of MyD88-linked innate receptors in skin and colon chemically induced carcinogenesis.

Role of MyD88 in colon and skin carcinogenesis. MyD88 is required for signaling through all Toll-like receptors except TLR3 and through the IL-1 receptor family. Studies by us and other have shown that expression of MyD88 is required for carcinogenesis in the skin, colon, and liver. In skin carcinogenesis, expression of MyD88 appears to be required for optimal papilloma formation in bothradiosensitive hematopoietic cells and radioresistant host cells. In order to investigate whether signaling through MyD88 in keratinocytes was important for tumorigenesis, we transformed in vitro keratinocytes with oncogeneic ras and we found that MyD88 expression in these cells was necessary for production of chemokines, metallo-proteases, and hematopoietic growth factors and also for the ras-mediated inhibition of keratinocyte differentiation. When grafted in vivo ras-transformed MyD-/- formed tumors much slower than wild type cells.. We identified that the lack of expression of MyD88 prevented the expression of the full ras-transformation program by blocking signaling through the interleukin-1 receptor. MyD88-/- mice are very susceptible to irradiation- and DSS-induced colitis due to defective epithelial mucosa repair possibly reflecting a role of microbial-derived ligands to stimulate TLR-dependent tissue homeostasis in physiological conditions and upon injury. Although highly resistant to all other model of carcinogenesis, MyD88-/- mice are very susceptible to AOM/DSS induced carcinogenesis, possibly due to their defective tissue repair after DSS-induced mucosal damage. IL-18-/- but not IL-1R-/- mice partially reproduce the phenotype of MyD88-/- mice: they are very susceptible to DSS colitis and AOM/DSS carcinogenesis.

Immunosuppression in tumor microenvironment induced by chronic inflammation

Viktor Umansky

German Cancer Research Center, Heidelberg, and University Hospital Mannheim, Germany

Melanoma is known for its poor response to current immunologic treatments. Insufficient anti-tumor reactivity could be due to the formation of a chronic inflammation represented by infiltrating leukocytes and soluble mediators, which lead to cancer progression. Using the ret transgenic mouse melanoma model that mimics human melanoma, we found in skin tumors and metastatic lymph nodes increased levels of inflammatory factors such as IL-1β, GM-CSF, and IFN-γ, which correlate with tumor growth. Moreover, Gr1+CD11b+ myeloid derived suppressor cells (MDSC) known to inhibit tumor reactive T cells were enriched in melanoma lesions and lymphatic organs during tumor progression. MDSC infiltration was associated with a strong TCR ζ-chain down-regulation. Co-culturing normal splenocytes with tumor-derived MDSC induced a decreased T cell proliferation and ζ-chain expression, verifying the MDSC immunosuppressive function. In addition to soluble chronic inflammatory mediators, tumor cells could induce immunosuppression by releasing of microvesicles (exosomes). We found that melanoma derived exosomes are able to convert Gr1+CD11b+ immature myeloid cells from normal mice into immunosuppressive cells producing high amounts of nitric oxide, expressing high arginase levels and being able to suppress T-cell proliferation.

Upon manipulation of the tumor microenvironment in melanoma bearing mice with the phosphodiesterase-5 inhibitor sildenafil, we observed reduced amounts of inflammatory mediators (IL-1β, VEGF, and GM-CSF) and immunosuppressive factors (nitric oxide and arginase-1) in association with decreased MDSC levels andimmunosuppressive function. These led to the restoration of ζ-chain expression in T cells and to a significant increased survival of treated mice. In addition, the chemomodulation with very low, non-cytotoxic and non-cytostatic doses of paclitaxel also led to a substantial retardation of melanoma progression associated with an inhibition of chronic inflammatory mediator production in melanoma lesions and with a reduction of MDSC immunosuppressive activity. Our data suggest that inhibitors of the immunosuppressive tumor microenvironment induced by chronic inflammation should be applied in conjunction with melanoma immunotherapies to increase their efficacy.

Institute of Microbiology speakers

The role of lectin-saccharide interactions in the evolution of cytokines

Bilej M.[1], Roubalová R.[1], Procházková P.[1], Šilerová M.[1], Lucas R.[2], Van den Bergh R.[2], De Baetselier P.[2], Beschin A.[2]

[1]Institute of Microbiology ASCR, v. v. i., Prague, Czech Republic, [2]Vlaams Interuniversitair Instituut voor Biotechnologie, VUB, Brussels, Belgium

Based on the assumption that invertebrates, like vertebrates, possess factors regulating responses to infection or wounding, studies dealing with the evolution of immunity have focused on the isolation and characterization of putative cytokine-related molecules from

invertebrates. Until recently, most of our knowledge of cytokine- and cytokine receptorlike molecules in invertebrates relies on functional assays and similarities at the physicochemical level. As such, a phylogenetic relationship between invertebrate cytokine-like molecules and vertebrate counterparts could not be convincingly demonstrated. Recent genomic sequence analyses of cytokine-related molecules suggest that invertebrate cytokine-like molecules and vertebrate factors do not have the same evolutionary origin. We propose instead that the convergence of function of invertebrate cytokine-like molecules and vertebrate counterparts involved in innate immune defenses may be based on similar lectin-like activities. Indeed, many cytokines possess lectin-like activity that may be essential for the expression of their full biological activities. The invertebrate pattern-recognition protein named coelomic cytolytic factor (CCF) and the mammalian cytokine tumor necrosis factor (TNF) share functional analogies that are based on similar saccharide recognition specificity. In particular, CCF and TNF have been shown to interact with ion channels on the surface of vertebrate cells via *N*,*N*'-diacetylchitobioselectin-like activity. In the present study, we show that CCFinduced membrane depolarization results in the release of TNF, IL-6 and nitric oxide (NO) by macrophages via nuclear factor-κB signaling. Interestingly, our data suggest that TNF contributes, through lectin-saccharide interaction, to the secretion of IL-6 and NO induced by CCF. This experimental non-physiological setting based on the interaction of an invertebrate defense lectin with vertebrate cells involved in the innate immune response may have highlighted an evolutionarily ancient mechanism ofmacrophage activation in vertebrates.

Tuning the immunity up and down with complexes of IL-2 and anti-IL-2 mAb

Jakub Tomala, Petra Votavová, Milada Šírová, Miloslav Kverka, Blanka Říhová, **Marek Kovář**

Department of Immunology and Gnotobiology, Institute of Microbiology ASCR, v.v.i., Prague, Czech Republic

IL-2 is approved by the FDA to treat metastatic renal cancer and melanoma. However, extremely short half-life and serious toxicities associated with IL-2 treatment are the main drawbacks. The biological activity of IL-2 can be strongly increased by association of IL-2 with anti-IL-2 mAb. IL-2/S4B6 mAb immunocomplexes are highly stimulátory mainly for NK and memory CD8+ T cells, while IL-2/JES6-1 mAb immunocomplexes solely for Treg cells. Here we show that both above mentioned IL-2 immunocomplexes are extremely potent in expanding activated naïve CD8+ T cells in vivo. IL-2 immunocomplexes expand activated naïve CD8+ T cells several hundred-fold times after four doses. On the contrary, free IL-2 given at the same dosage shows negligible activity. Importantly, activated naïve CD8+ T cells expanded by IL-2 immunocomplexes form stable robust population of functional memory cells. Using radioactively labeled IL-2, we provide for first time direct evidence that IL-2 immunocomplexes have much longer halflife than free IL-2. Finally, we demonstrate that IL-2/S4B6 mAb immunocomplexes possess considerable anti-tumor activity while IL-2/JES6-1 mAb immunocomplexes can be used to enormously expand Treg cells which are able to protect the mice from induced acute colitis and also to inhibit rejection of syngeneic tumor cells expressing xenogenic protein.

Oral treatment with antigens from *Parabacteroides distasonis* protects mice from experimental colitis by oral tolerance induction

Kverka, M.[1], Zákostelská, Z.[1], Klimešová, K.[1], Hudcovic, T.[2], Hrnčíř, T.[2], Rossmann, P.[1], Tlaskalová-Hogenová, H.[1]

Department of Immunology and Gnotobiology, Institute of Microbiology ASCR, [1]Vídeňská 1083, Prague, and [2]Nový Hrádek, Czech Republic

Intestinal inflammation in Inflammatory Bowel Disease (IBD) results from aberrant mucosal immune responses to nonpathogenic gut microbiota. Here, we show that oral pre-treatment of BALB/c mice with antigens from particular commensal microbe, *Parabacteroides distasonis*, significantly reduces the severity of intestinal inflammation in dextran sulphate sodium (DSS)-induced model of colitis. This treatment significantly increased the number of regulatory T cells in mesenteric lymph nodes of treated mice and prevented DSS-induced increases in several pro-inflammatory cytokines in the gut mucosa. This protective effect was significantly reduced when the antigens were administered together with the strong mucosal adjuvant, cholera toxin, and could not be achieved by parenteral administration of this antigen. Moreover, the protective effect was not observed in mice with severe combined immunodeficiency or in immunocompetent BALB/c mice, in which Tregs were depleted by anti-CD25 antibody. Our results suggest that components derived from the commensal bacterium, *P. distasonis*, protect from intestinal inflammation by mechanisms including induction of oral tolerance, and therefore may be useful in the development of new therapeutic strategies for chronic inflammatory disorders such as IBD.

The role of the ileal Peyer's patches and bone marrow in B cell development in swine

Marek Šinkora

Laboratory of Physiology, Immunity and Ontogenesis of Gnotobionts, Department of Immunology and Gnotobiology, Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic, Nový Hrádek, Czech Republic

Pigs are traditionally categorized into group of animals that use Gut Associated Lymphoid Tissues (GALT) for generation of B cell repertoire. This group include chicken that use bursa of Fabricius, rabbit that use sacculus rotundus (or appendix) and other ungulates including swine that use Ileal Peyer's Patches (IPP). Our recent studies disprove this categorization by showing that at least in swine (and probably in other ungulates), IPP are not a significant source of B cells, are not required for maintenance of the systemic B cell pool and are not a site of primary B cell lymphogenesis. According to finding that IPP are a secondary lymphoid tissue we have extended those studies by showing that porcine B cells are developed in the bone marrow, which is the primary lymphogenetic organ also for mouse and human.

Tumor microenvironment as a target for selective immunotherapy

Luca Vannucci[1], Oleksandr Chernyavskiy[2], Jiří Křížan[1], Lenka Rajsiglová[1], Monika Burocziová[1], Renata Štěpánková[1], Jiří Janáček[2], Pavel Rossmann[1], Klára Klimešová[1], Veronika Poláková[3], Jana Slyšková[3], Ludmila Vodičková[3], Miroslav Levý[4], Lide Arana[5]

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A model for studying cancer and inflammation relationships is furnished by the bowel, both in healthy and disease conditions. Observations in GF rats has suggested suggest that in these animals the different antigenic challenge, absence of the "physiologic inflammation" elicited by the presence of commensal microflora in the gut (threshold of immune regulation), together with the different metabolism of the intestinal content, may influence both structure of the bowel and systemic immunity. The different stromal architecture may create different niches for colonocyte and immune cell maturation influencing local and systemic immunity. Thanking the new technical progresses in confocal imaging, we went to evaluate both in the living animal, fresh samples and fixed samples the variations induced on the tissue architecture by induced cancer as well as induced inflammation, following the evolution of these processes. Interestingly, since the inflammation was re-discovered as an important multifaceted process accompanying cancer evolution, we firstly found alterations of cancer stromal organization similar to alterations induced in the colon inflammatory models (colitis induced by dextan-sulfate) *in vivo*. Moreover, the comparison with germ-free animals suggested an important role of proinflammatory products in shaping the tissue stromal structure. Animals induced to colon inflammation (DSS) and subsequently challenged with a carcinogen (AOM) developer tumors with important anticipation than in the treated with DSS-only or AOM-only. Germfree animals developed tumors at a lower extent than animals with intestinal bacteria. We found correlations within the immunological conditions, oncogene products levels and morphological changes, with possible important role of TGF-beta. K-ras, TGF-beta and VEGF expression differently associated to inflammation and cancer in different colon segments. Therefore, it is possible that molecules produced by and delivered within the microenvironment may be elicited and may drive the evolution of a pathological state. This makes the tumor microenvironment (as other pathological microenvironments) and its structures suitable for new therapeutic approaches directed to target *in situ* the components orchestrating the illness development. Nanotechnologies can permit a very wide application for trying to control and/or interfere with pathogenic mechanisms involving the dynamic interplay between cells and stroma in the microenvironment. Preliminary results in a mouse melanoma model resulted very promising, showing cytoplasmic accumulation of specifically designed physiological nanoparticles inside the tumor cells.

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