CLINICAL IMMUNOLOGICAL AND IMMUNOGENETICAL ASPECTS OF NEUROLOGICAL DISEASES

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PhD Theses

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I. INTRODUCTION

The term *immunity* is derived from the Latin word *immunitas,* which referred to the protection from legal prosecution offered to Roman senators during their tenures in office. Today, in life sciences not only does ***immunity*** cover recognition and destruction of infectious agents, noninfectious foreign substances and tumor cells, but also the maintenance of healthy tissues, leaving them intact. Defense against foreign cells and substances, viruses and tumor cells is mediated by the early, rapid, non-specific reactions of innate (also called natural or native) immunity and the later, specific responses of adaptive immunity, the two functional arms of the immune system.

Natural killer (NK) cells are a principal component of the innate immune system, able to rapidly produce abundant cytokines, mainly interferon-gamma (IFN-γ), and lyse target cells by means of cytotoxicity. They express CD56 (cluster of differentiation) on their surface. **Perforin** (pore forming in target cell membranes leading to cell lysis) and **Fas/Fas-ligand (FasL)** (inducing apoptosis) pathways are the two major mechanisms of cellular cytotoxicity.

T lymphocytes are one of the major cellular components of the adaptive immune system. Specific recognition of different antigenic determinants is mediated by their **T cell receptor (TCR, CD3)**. T cell receptors comprise an essentially unlimited repertoire of variants, each variant expressed on a different cell, which can altogether recognize virtually any molecule. The TCR is a heterodimer consisting of an α and a β transmembrane polypeptide chain, each consisting of one variable (V) domain and one constant (C) domain. The V regions of the TCR α and β chains contain the hypervariable or complementarity-determining regions (CDRs), which specifically recognize peptide-MHC (major histocompatibility complex) complexes. In the α and β chains of the TCR, the third hypervariable regions are composed of sequences encoded by V and J (joining) gene segments (in the α chain) or V, D (diversity), and J segments (in the β chain). T cells may be $CD8^+$ cytotoxic T cells, killing infected cells. $CD4^+$ T helper (Th) cells activate other cells of the immune system, which kill microorganisms or secrete antibodies. Immune cells secrete cytokines that mediate many functions of these cells. **T helper type 1 (Th1) or pro-inflammatory cytokines** – IFN-γ, interleukin (IL)-12 – enhance cellular immune responses, cell-mediated cytotoxicity, and activate macrophages. **T helper type 2 (Th2) cytokines** called anti-inflammatory cytokines – IL-4, IL-5, IL-6, IL-10, IL-13 – generate humoral immune response, and antibody production. Recently, other pro-inflammatory cytokines, **IL-23 and IL-17** have been indicated to play an important role in the establishment of autoimmune diseases, and T cells producing IL-17 have been dubbed Th17 cells. One of the Th17 differentiation pathways is IL-23-dependent and IL-23 is necessary for the survival and expansion of Th17 cells; this effect is mediated through the IL-23 receptor (IL-23R) signaling pathway. For the full activation of T cells, besides the antigen-specific stimulation through the TCR, a second signal called co-stimulation is necessary. The termination of activation is a very important mechanism in autoimmune tolerance, protection against autoimmune diseases. This termination is mainly mediated by **CTLA-4 (cytotoxic T-lymphocyte-associated antigen-4)** expressed on the surface of T cells upon activation, which intervenes in the co-stimulatory pathway.

A small subset of T cells, called **γδT cells** have γδTCRs consisting of a γ-chain combined with a δ-chain, composing a distinct T cell population from αβT cells. γδT cells are proposed to bridge the innate and adaptive immune responses, since they are able to produce great amounts of IFN-γ in a short period of time, which is characteristic to cells of the innate immune system. The Vδ2 subset, expressing a δ2TCR variable chain, represents the majority of adult γδT cells mainly with a cytotoxic property.

A small percentage of αβT cells express molecules specific to NK cells, such as CD56 in addition to the TCR (CD3), termed **natural killer T**, *ie.* NKT cells. This population, which has been suggested to play an important role in tumor rejection, protection against autoimmunity and infectious agents, also bridges the innate and the adaptive immune responses. NKT-like cells represent a small lymphocyte subpopulation that has important immunoregulatory functions. NKT cells are categorized into several distinct subsets principally based on their consistent TCR repertoire. NKT cells expressing an invariant Vα24-JαQ TCR α-chain are referred to as **invariant NKT (iNKT) cells**. Emerging data indicate the functional diversity of human iNKT cells and their involvement in tumor immunity and autoimmunity. Invariant Vα7.2-Jα33 TCR expressing cells have been named **mucosal-associated invariant T (MAIT) cells** relating to their preferential location, the gut lamina propria. MAIT cells express a restricted Vβ2 and Vβ13 driven by the selecting antigen. Their anti-inflammatory role has been proposed, for the invariant Vα7.2-Jα33 TCR of MAIT cells was found to be present in autoimmune lesions of both the central and peripheral nervous system, which correlated with the expression of IL-4.

Key information:
iNKT: invariant natural killer T cells expressing an invariant Vα24-JαQ TCR α-chain, playing a substantial role in protection against tumors and autoimmunity
MAIT: mucosal-associated invariant T cells expressing an invariant Vα7.2-Jα33 TCR
NK cells: natural killer cells: $CD56^+CD3^-$
T cells: $CD3^+$
NKT-like cells: natural killer T-like cells: $CD3^+CD56^+$
LAR: leukocyte antisedimentation rate: detects activation of leukocytes
CTLA-4: cytotoxic T-lymphocyte-associated antigen-4: termination of T cell activation
IL-23R: interleukin-23-receptor: mediates Th17 cell differentiation. Th17 cells (producing IL-17) play an important role in the establishment of autoimmune diseases

Multiple sclerosis (MS) is a chronic, progressive, demyelinating disease of the central nervous system, with a wide range of varying neurological symptoms. There are about 2-3 million people suffering from MS in the world, in Hungary the number of MS patients is approximately 6,000-10,000. Despite a large body of research, the exact etiology of MS remains unclear. Four different pathological subtypes of MS exist, suggesting the heterogeneous nature of MS pathogenesis. According to the current, autoimmune hypothesis, the demyelination of plaques is mediated by mainly autoimmune $CD4^+$ Th1 cells recognizing central nervous system (CNS) autoantigens due to delayed-type or type IV hypersensitivity. MS is a complex genetic disease mediated by interaction of several genes and environmental factors. It is assumed today that a tremendous number of different genes play a role in the risk of catching the disease (polygenetic inheritance).

Stroke – cerebrovascular accident – has a high incidence worldwide, in addition to being the third leading cause of death, many survivors of stroke have to adjust to a life with varying degrees of disability. In Hungary approximately 40,000 patients are admitted to hospital with the diagnosis of stroke and stroke mortality is extremely high in Central-Eastern European countries. While direct neurological deficits cause early deaths, infectious complications, particularly pneumonia and urinary tract infections, prevail in the postacute phase of stroke contributing to the poor outcome, which may suggest early alteration of immune responses. Recently, a loss of T cells in the peripheral blood of patients with acute ischemic stroke within 12 hours from onset of stroke symptoms, was revealed, which gradually normalized.

II. AIMS OF THE STUDIES

Here, we investigated molecular bases of neurological diseases focusing mainly on molecules and mechanisms involved in regulating autoimmune tolerance *ie.* protecting against autoimmune diseases. We were particularly interested in the role of innate T cells in immune responses within the CNS and the neurogenetical background of CNS autoimmune diseases, particularly multiple sclerosis.

A. The role of innate T cells in diseases affecting the central nervous system

In the recent years, phenotypically and functionally similar iNKT and MAIT cells have been suggested to participate in immunoregulation of autoimmunity and immune surveillance of tumors.

We have recently found that Vα24-JαQ iNKT cells were absent in CNS plaques of MS, while conventional T cells expressing non-invariant Vα24$^+$ TCR were present. It was not clear whether this selective absence of iNKT cells was related to the disease or the special immunoregulation and antigens within the CNS. Therefore, we addressed:

1. Do iNKT cells infiltrate tumors within the CNS similarly to tumors outside the CNS?
2. Can we detect other invariant T cells in tumors with a special emphasis on MAIT cells, which may possess similar functions to iNKT cells?
3. If MAIT cells are present in tumors, what is the inflammatory environment? MAIT cells have been suggested to produce Th2 cytokines, but the similarity to iNKT cells and recent murine data suggested that the MAIT subset may be functionally heterogeneous as well.
4. What is the relation of MAIT and NKT-like cells in tumors? Do MAIT cells express CD56?
5. Do CD56$^+$ MAIT cells participate in anti-tumor immune responses?

Atherosclerosis and acute ischemic stroke are accompanied by immune responses. We may suspect that early changes in immune functions may be related to post-stroke infections resulting in poor outcome and high mortality of stroke. Therefore, we examined innate T cells and NKT-like cells in the early phase of acute ischemic stroke. We also explored activation of leukocytes and its relation to post-stroke infections:

6. Are innate T cells including CD3$^+$CD56$^+$ NKT-like cells affected by acute ischemic events?
7. What is the relation of leukocyte activation to post-stroke infections?
8. Does post-stroke leukocyte activation depend on the duration of ischemia and the extent of infarct?
9. Can we use simple bed-side tests to predict outcome and susceptibility for post-stroke infections?

B. Association of multiple sclerosis with polymorphisms of genes involved in shaping immune responses and regulating autoimmune tolerance

CTLA-4 is an important molecule to terminate immune responses and thus to prevent autoimmunity. Data about association of MS with *CTLA4* polymorphisms are conflicting. By using a large number of patients, here we examined:

10. Are polymorphisms of the *CTLA4* gene associated with multiple sclerosis?
11. Do polymorphisms of the *CTLA4* gene influence expression of co-stimulatory molecules important in terminating or shaping immune responses?

A novel functional Th cell subpopulation (Th17) has recently been described as the main autoimmune T cell subset. One of the Th17 differentiation pathways is IL-23-dependent. IL-23 is necessary for the survival and expansion of Th17 cells mediated via the IL-23 receptor (IL-23R) signaling pathway. Association of polymorphisms of *IL23R* gene has recently been suggested in autoimmune inflammatory bowel disease. Therefore, we examined:

12. Are polymorphisms of the *IL23R* gene associated with multiple sclerosis?
13. Is *IL23R* a shared autoimmunity gene?

MS is a complex genetic disease mediated by interaction of several genes and environmental factors. In addition, regulation of gene expression is controlled through the combinatorial action of multiple transcription factors. Therefore, we performed a gene network analysis to examine complex autoimmune processes underlying the pathogenesis of MS:

14. Are there gene expression networks disregulated in multiple sclerosis?

III. EXPERIMENTS

A. THE ROLE OF INNATE T CELLS IN DISEASES AFFECTING THE CENTRAL NERVOUS SYSTEM

1. Invariant Vα7.2-Jα33 TCR is expressed in human kidney and brain tumors indicating infiltration by mucosal-associated invariant T (MAIT) cells

The anti-tumor response of human iNKT cells is well established. A novel T cell subset, mucosal-associated invariant T (MAIT) cells, possess similar regulatory properties to iNKT cells in autoimmune models and disease, but data about other functions of human MAIT cells are very limited, partly due to the absence of clonotypic antibodies. In addition, the selective absence of iNKT cells has previously been indicated in MS plaques in contrast to autoimmune inflammatory demyelinating lesions of the peripheral nervous system and MAIT cells. To address whether such deficiency of iNKT cells is related to the CNS environment or MS, we examined the presence of four known T cells with invariant αTCRs including that of iNKT cells in tumors within and outside of the CNS. The presence of sequences of Vα24-JαQ (iNKT), Vα7.2-Jα33 (MAIT), Vα4-Jα29 and Vα19-Jα48 TCRs was investigated in 19 biopsy samples of human kidney cancers and brain tumors by using RT-PCR SSCP (single-strand conformation polymorphism) clonality assay, identifying the amplified and electrophoretically separated particular sequences by hybridization with specific probes.

The MAIT clonotype was identified and co-expressed with iNKT clonotype in half of the tumors. In contrast, invariant Vα4 and Vα19 T cell clonotypes were not present in tumors. Vβ expression of Vα7.2-Jα33 MAIT cells with a restricted Vβ2 and Vβ13 TCR usage was further analyzed in tumor samples containing MAIT cells, as well as their antigen-presenting molecule, MR1 expression and pro- and anti-inflammatory cytokine environment. All kidney tumors with MAIT αTCR also expressed Vβ2 and Vβ13, in contrast to brain tumors, suggesting that MAIT cells in CNS tumors may express other Vβ chains as well. All tumors positive for MAIT invariant TCR express the antigen presenting molecule MR1, indicating that MAIT cells can be locally re-activated. Indeed, a high percentage of infiltrating T cells was $CD8^+$ and expressed HLA-DR suggesting activation. The clonal presence of MAIT cells in tumors correlated with the expression of pro-inflammatory cytokines but no IL-4, IL-5 and IL-10, suggesting that a pro-inflammatory subset of human MAIT cells may exist. We also

examined CD56 expression of MAIT cells both in tumor samples and in the peripheral blood of tumor patients. Although the MAIT αTCR was identified in both peripheral $CD56^+$ and $CD56^-$ subsets, tumor infiltrating lymphocytes were CD56 negative.

Our data imply that a $CD56^-$ subset of MAIT cells may participate in pro-inflammatory tumor immune responses similarly to iNKT cells. In addition, MAIT cells may have a pro-inflammatory T cell subset, similarly to human iNKT and murine MAIT cells. Our data also suggest that the selective absence of iNKT cells in CNS plaques of MS may be specific to the disease and not related to the CNS environment.

2. Impaired function of innate T lymphocytes and NK cells in the acute phase of ischemic stroke

While direct neurological deficits cause early deaths, infectious complications prevail in the postacute phase of stroke contributing to the poor outcome. The increased susceptibility to infections after stroke may suggest early alteration of immune responses, thus immunodepression induced by stroke has been proposed. The few animal and human studies all addressed the rapid changes in the adaptive arm of the immune system, mainly T cells. We analyzed rapid changes in immunological functions of cells of the innate immunity or lymphocytes bridging the innate and the adaptive arms of the immune system, all capable of shaping subsequent immune responses through rapid production of cytokines, and/or cytotoxicity. The analyzed cell subsets were Vδ2 T cells, $CD3^+CD56^+$ natural killer T (NKT)-like cells and $CD3^-CD56^+$ NK cells. Their frequencies, cytokine production, intracellular perforin and surface Fas ligand (FasL) expression were measured in 28 patients' peripheral blood obtained within 6 hours and also after 72 hours of ischemic stroke, by flow cytometry including cytometric bead array. NK cytotoxicity was also sequentially determined applying a non-radioactive, colorimetric cytotoxicity assay. The paired samples were compared both with each other and with 20 healthy controls.

Percentages of Vδ2, NKT-like and NK cells at 6 and 72 hours after stroke were constant and similar to percentages in healthy subjects. In contrast, pro-inflammatory intracellular IFN-γ expression by Vδ2 T cells, NKT-like cells and NK cells and IFN-γ production by isolated NK cells in culture were low at 6 hours and reached the level of healthy subjects' by 72 hours after stroke. Anti-inflammatory IL-4, IL-5 and IL-10 production of NKT-like and NK cells was not altered. Intracellular perforin expression by Vδ2 T cells, NKT-like cells and NK cells, and NK cytotoxicity were low at 6 hours and reached the level of healthy subjects by 72 hours.

According to our results, pro-inflammatory and cytotoxic but not anti-inflammatory responses of NK, NKT-like and Vδ2 T cells become acutely deficient in ischemic stroke, which may contribute to an increased susceptibility to infections.

3. Deficient leukocyte antisedimentation is related to post-stroke infections and outcome

Patients with stroke are more susceptible to infections suggesting possible deficiencies of early immune responses, particularly of leukocytes. Here, we examined whether post-ischemic activation of leukocytes is related to duration of ischemia and extent of infarct. We also addressed, if disregulated leukocyte activation might be related to post-stroke infection and worsen outcome. We used leukocyte antisedimentation rate (LAR) to detect activation of leukocytes and correlated LAR with clinical and laboratory parameters. LAR test is performed similarly to Westergreen test, determination of leukocyte counts is also needed for calculation of LAR. An additional aim was to test simple bed-side investigations in predicting outcome and susceptibility for post-stroke infections early.

LAR, a simple test to detect activation of leukocytes was serially examined and correlated with blood level of S100β related to extent of infarct, procalcitonin indicating infection and outcome in patients with acute ischemic events. Venous blood samples were taken from 61 healthy volunteers and 49 patients with acute ischemic events: 38 patients with acute ischemic stroke, AIS, and 11 patients with transient ischemic attack, TIA where symptoms disappear in 24 hours and cranial CT scan does not indicate infarct. Sampling was done within 6 hours, at 24 and 72 hours after onset of symptoms. LAR was significantly higher in acute ischemic events within 6 hours after onset of stroke regardless of post-stroke infections. In addition, elevation of LAR was delayed and attenuated in TIA in contrast to AIS and we also observed a positive correlation between LAR and S100β at 72 hours after the onset of ischemic stroke both indicating that the extent of tissue injury correlates with the magnitude of innate immune responses. Importantly, a deficiency in early elevation of LAR was associated with post-stroke infections and a poor outcome measured by Glasgow Outcome Scale in AIS.

We conclude that an early activation of leukocytes indicated by elevation of LAR is characteristic of acute ischemic cerebrovascular events. A delayed and ameliorated leukocyte activation represented by LAR is characteristic to TIA in contrast to definitive stroke. Our data suggest that acute activation of leukocytes, which has been regarded detrimental so far, serves also to prevent post-stroke infections. Our data imply that concept about the post-ischemic role of leukocytes should be changed and dissected: recruitment of leukocytes in the CNS may be damaging but should be separated from the systemic activation, which may prevent post-stroke infections. A disregulated early immune response or deficient leukocyte activation may result in an increased susceptibilty to infections in some patients with stroke.

B. ASSOCIATION OF MULTIPLE SCLEROSIS WITH POLYMORPHISMS OF GENES INVOLVED IN SHAPING IMMUNE RESPONSES AND REGULATING AUTOIMMUNE TOLERANCE

4. Multiple sclerosis and the *CTLA4* autoimmunity polymorphism CT60: no association in patients from Germany, Hungary and Poland

For the full activation of T cells, besides the antigen-specific stimulation through TCR, a second signal called co-stimulation is necessary. Both APC and T cells will be activated during co-stimulation, a sequence of ligand-receptor interactions on the surface of both cells. Such interactions are required also for the termination of activation, a mechanism important in autoimmune tolerance. This termination is mainly mediated by CTLA-4 (cytotoxic T-lymphocyte-associated antigen-4) expressed on the surface of T cells upon activation.

Polymorphisms in the *CTLA4* gene region have been associated with susceptibility to autoimmune diseases. The recently described single nucleotide polymorphism *CT60*, located in the 3' untranslated region (3'UTR) of *CTLA4* is associated with Graves' disease, thyroiditis, autoimmune diabetes and other autoimmune diseases, however, its role in multiple sclerosis (MS) susceptibility has been controversial. Therefore, we conducted a case-control association study in a large number of German, Hungarian and Polish MS patients and regional control individuals for the *CTLA4 CT60* and *+49A/G* polymorphisms by using PCR methodology. We also performed haplotype analysis. Besides, we examined functional consequences *ie.* genotype differences in the expression of CTLA-4 and ICOS (inducible co-stimulatory molecule). ICOS is also important in autoimmune tolerance due to its role in Th2-mediated anti-inflammatory responses and susceptibility to experimental autoimmune encephalomyelitis (EAE), the animal model of MS is related to locus containing genes for both CTLA-4 and ICOS. Splice variants of CTLA-4 modify expression of ICOS in such models.

However, we found no significant association of these polymorphisms or respective haplotypes with MS, even when our data were extended with previously published results in a metaanalysis thus evaluating 1228 MS and 1440 controls. No association of *CT60* genotypes with T cell expression of ICOS and CTLA-4 after *in vitro* stimulation was detected. In summary, our data using a large number of cases and controls contradict to a major effect of *CTLA4* in MS susceptibility.

5. 3'UTR C2370A allele of the IL-23 receptor gene is associated with relapsing-remitting multiple sclerosis

Besides Th1 type cytokines, other pro-inflammatory cytokines, IL-23 and IL-17 have been indicated to play an important role in the establishment of autoimmune diseases, and T cells producing IL-17 have been dubbed Th17 cells. One of the Th17 differentiation pathways is IL-23-dependent and IL-23 is necessary for the survival and expansion of Th17 cells; this effect is mediated through the IL-23 receptor (IL-23R) signaling pathway. The IL-23/IL-17 cytokine axis has been suggested to play an important role in the development of several autoimmune diseases including multiple sclerosis.

We compared the prevalence of C2370A single nucleotide polymorphism (SNP) in the 3' untranslated region (3'UTR) of the IL-23 receptor gene (*IL23R*) of 223 patients with relapsing-remitting multiple sclerosis (RRMS) to 200 healthy controls by PCR technique. The A2370A genotype was significantly over-represented among patients with RRMS (10.8%) and RRMS exhibiting oligoclonal bands in the cerebrospinal fluid (12.9%) when compared to healthy subjects (5.50%). Multiple regression analysis revealed that presence of AA genotype provides a two-fold risk for the development of multiple sclerosis (OR=2.072, 95% CI: 0.988-4.347, $p<0.05$) and presence of oligoclonal bands in the CSF (OR=2.554, $p=0.03$). We did not find significant differences when female patients or HLA-DRB1*1501 positive/negative patients were separately analyzed and compared to controls. Nor did we find association in secondary progressive cases, athough the simple size was small (n=45).

Besides suggesting association with MS, these data indicate that *IL23R* represents a novel shared susceptibility gene as its association with several autoimmune diseases including inflammatory bowel disease (IBD) and psoriasis has recently been verified.

6. Aberrant transcriptional regulatory network in T cells of multiple sclerosis

Although several data indicated altered gene expression profile in MS using microarrays, transcriptional networks, which can regulate a number of genes, have not been examined in MS. In addition, genetic differences may also contribute to altered expression of genes. The concordance rate of monozygotic twins is approximately 30 %, while it is less than 5 % for dizygotic twins, suggesting the involvement of not a single but multiple susceptibility genes in the pathogenesis of MS. In addition, regulation of these multiple susceptibility gene expressions is controlled again through the combinatorial action of multiple transcriptional factors. Therefore, gene network analysis is necessary to evaluate the complex autoimmune processes underlying the pathogenesis of MS.

Therefore, we studied gene expression profile of purified $CD3^+$ T cells isolated from Hungarian monozygotic MS twins by DNA microarray analysis and performed gene network analysis. Three pairs were concordant, while one pair was discordant for MS. By comparing the three concordant and one discordant pairs, we identified 20 differentially expressed genes (DEG) between the MS patient and the genetically identical healthy subject. Molecular network of 20 DEG analyzed by KeyMolnet, a comprehensive information platform, indicated the close relationship with transcriptional regulation by the Ets transcription factor family and the nuclear factor NF-κB.

This novel bioinformatic approach proposes the logical hypothesis that aberrant regulation of the complex transcriptional regulatory network contributes to development of pathogenic T cells in MS.

V. SUMMARY OF THESES

1. MAIT and NKT cells are the only known invariant T cells infiltrating brain and kidney tumors.
2. Since NKT cells are present in CNS tumors, their absence in MS plaques is disease-specific and not related to the CNS environment.
3. MAIT cells may have a pro-inflammatory subset, which infiltrates tumors.
4. Brain and kidney tumors differ in infiltrating T cell and MAIT cell subsets: brain tumor infiltrating MAIT cells may express additional TCRβ to Vβ2 and Vβ13.
5. MAIT and NKT cells in tumors do not express CD56 although both $CD56^+$ and $CD56^-$ subsets are present in the peripheral blood even in patients with cancer.
6. The percentages of particular innate lymphocytes, Vδ2, NKT-like and NK cells do not change in the acute phase of ischemic stroke in contrast to the reported decrease of adaptive T cells.
7. In contrast to unaltered frequency, an acute functional deficiency of innate lymphocytes occurs in the acute phase of ischemic stroke, within 6 hours: pro-inflammatory cytokine production, expression of perforin and NK cytotoxicity are decreased, while there is no change in production of Th2 cytokines and Th2-related ICOS expression. We may hypothesize that such early deficiency or its disregulated normalization may substantially influence susceptibility to infections similarly to animal models of cerebral ischemia.
8. Activation of leukocytes represented by elevation in LAR happens within hours after onset of ischemic stroke.
9. Although LAR was elevated in TIA compared to healthy subjects, it was delayed and ameliorated compared to definitive ischemic stroke.
10. Decreased activation of leukocytes reflected by a deficient elevation of LAR may predispose to post-stroke infections and predict worse outcome.
11. Concept about uniformly harmful post-ischemic role of leukocytes should be changed and dissected: recruitment of leukocytes to the ischemic brain may be damaging by amplifying brain injury. However, systemic activation of leukocytes plays an important role in preventing post-stroke infections.
12. A positive correlation exists between LAR and S100β on the 3rd post-stroke day indicating a relationship between extent of infarct and innate immune responses.
13. There is no significant effect – if at all – of the *CT60*G* allele on susceptibility to MS compared to other autoimmune diseases. No genotype-dependent expression differences in CTLA-4 and ICOS by T cells were found. Taken together, our data, together with previously published studies, suggest lack of association of this common autoimmune gene with multiple sclerosis.
14. A genetic association exists between RRMS and the C2370A polymorphism (AA) of the *IL23R* gene: the susceptible genotype provides a two-fold risk for developing MS.
15. *IL23R* may represent a novel shared autoimmunity gene.
16. Aberrant regulation of the complex transcriptional regulatory network contributes to the development of pathogenic T cells in MS.

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