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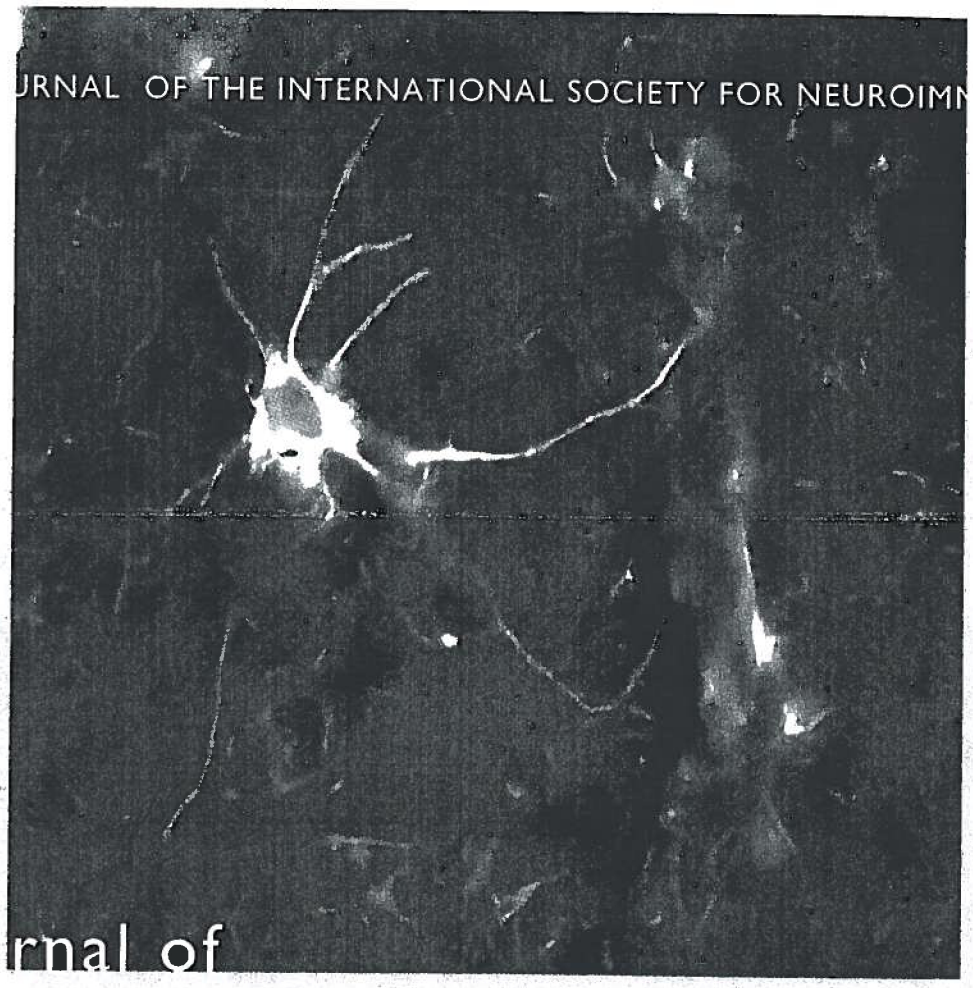

Neuroimmunology

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innate immune responses, particularly in the CNS. They could thereby possibly contribute to adverse effects when MS patients that use statins would suffer from bacterial or viral infections.

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The multiple sclerosis susceptibility allele in TNFRSF1A creates a new RNA isoform that results in a truncated TNFRSF1A protein

Ottoboni Linda*[,1], Healy Brian[1], Wood Irene[1], Hafler David[2], De Jager Philip[1]

[1]*Brigham and Women's Hospital and Harvard Medical School, Boston, United States*; [2]*Yale School of Medicine, New Haven, United States*

Multiple sclerosis is an inflammatory and neurodegenerative disease with an important genetic component. Our meta-analysis of genome-wide association scans (GWAS) in MS identified a novel susceptibility locus in TNFRSF1A (rs1800693, $P = 1.6 \times 10{-}11$). Disruption of TNF signaling severely affects human and murine demyelinating neuroinflammatory diseases.

The rs1800693 polymorphism is intronic and is located near the exon–intron 6 boundary. We found that this polymorphism gives rise to a new splice variant isoform of TNFRSF1A which lacks exon 6 and is predicted to produce a truncated protein deficient in its transmembrane and intracellular component. The level of RNA expression of the truncated isoform (delta 6) was measured in PBMCs of 84 genotyped healthy subjects from the PhenoGenetic Project and displays a robust linear correlation with the frequency of the susceptible allele (using the PLINK analysis toolkit): while the full-length isoform is present in all individuals, the delta 6 isoform is only found in the presence of the MS susceptibility allele. The level of expression of this novel isoform is also upregulated in PBMC of subjects with one or two copies of the risk allele after PMA stimulation. The delta 6 isoform of TNFRSF1A does not appear to affect either (1) the levels of soluble TNFRSF1A and TNFRSF1B in serum samples from healthy ($n = 265$) and MS subjects ($n = 282$) or (2) the surface expression of TNFRSF1A on monocytes and granulocytes as assessed by flow cytometry. In vitro experiments show cytoplasmic intracellular expression and secretion of the delta 6 isoform of TNFRSF1A.

Here, we present evidence of one functional consequence of a high frequency (45%) MS susceptibility allele within TNFRSF1A: this variant leads to the production of a novel TNFRSF1A RNA isoform and production of a truncated protein.

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The role of activated microglia in LPS-induced neuroprotection in an aseptic brain injury model

Chen Zhihong*, Jalabi Walid, Dutta Ranjan, Trapp Bruce

Cleveland Clinic, Cleveland, United States

Therapeutics that protect against neuronal death will have many applications for treating acute and chronic diseases of the central nervous system (CNS) such as neurodegenerative diseases that include multiple sclerosis (MS). We have previously demonstrated that multiple low dose i.p. injections of LPS (lipopolysaccharide) in mice lead to global activation of microglia, reduced inhibitory GABAergic pre-synaptic components, and the induction of the pro-survival signaling pathways in neurons. Here, by using an aseptic cryogenic brain injury model, we study the contribution of this neuroprotective effect offered by microglia.

By immunohistochemistry, we show that LPS-activated microglial cells gather around the penumbra of the lesions caused by experimental brain injury. These cells demonstrate phagocytic capability and actively participate in clearing of hemorrhagic debris, which are characteristics of anti-inflammatory tissue-repairing "M2" phenotype macrophages. A further analysis of the dataset obtained using DNA microarray technology has revealed that a cluster of genes that are related to the M2 subtype has indeed increased their transcription after LPS injections. In addition, LPS preconditioned mice have reduced volume of lesion size after brain injury; and these animals correspondingly perform better on "rotarod", the equipment used to test locomotor behavioral functions.

Conclusively, we demonstrate that LPS preconditioning leads to increased M2-microglia and decreased neuronal loss after brain damage. The molecular events that elicit this neuroprotective action possibly proffered by activated microglia are now under investigation.

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The role of the complete Freund's adjuvant and neutrophils in actively induced Lewis rat EAE

Rigolio Roberta*[,1], Chiorazzi Alessia[1], Meregalli Cristina[1], Canta Annalisa[1], Carozzi Valentina Alda[1], Rodriguez-Menendez Virginia[1], Avezza Federica[1], Crippa Luca[2], Oggioni Norberto[1], Cavaletti Guido[1]

[1]*Università Milano-Bicocca, Monza, Italy*; [2]*Veterinary Pathology Labs, ISTOVET, Monza, Italy*

Actively induced Lewis rat Experimental Autoimmune Encephalomyelitis (EAE) is a highly reproducible model for investigating the cellular and molecular mechanisms involved in leukocyte central nervous system invasion. The disease is induced by the subcutaneous injection of guinea pig Myelin Basic Protein (gpMBP) resuspended in incomplete Freund's adjuvant added with Mycobacterium Tuberculosis (CFA). The disease becomes clinically evident 9–10 days post EAE induction (p.i.) and neurological signs peak on days 13–14 with extensive leukocyte inflammation mainly located in the spinal cord (S.C.).

On day 14 p.i. we previously observed by flow cytometry the massive presence of a cellular population other than lymphocytes in the S.C. (i.e. the target organ) as well as in the spleen (i.e. imunoregulatory organ) of EAE rats.

We hypothesized that this population could be composed of polymorphonuclear cells and we wondered if its presence was related to CFA use or if it had to be referred to the disease itself. Thus, we focused our attention on the characterization of the immune system cellular components present in the S.C. and in the spleen of CFA and EAE animals on day 14 p.i.

We demonstrated that the cell population observed in the S.C. was mainly composed of neutrophils which invaded the central nervous system together with lymphocytes only in EAE animals; in fact, no leukocyte infiltration was observed in CFA treated rats.

The remaining cellular changes could be observed to the same extent in both CFA and EAE animal spleen. In fact, in both CFA and EAE spleen we observed a red pulp hyperplasia together with a white pulp lymphoid hypoplasia while the flow cytometry confirmed a decrease in T cell and that the relevant cellular population previously observed was mainly composed of neutrophils.

We also observed that CFA administration and EAE induction similarly increased neutrophils and monocytes absolute number vs controls, while a significant reduction in circulating lymphocytes was reported in EAE animals vs. CFA treated ones.

CFA was clearly responsible for spleen cellular changes as well as neutrophil and monocyte number increase among circulating cells. Nevertheless, the presence of infiltrating leukocytes in the spinal cord and the difference in circulating lymphocytes between CFA and EAE animals were both peculiar events occurring in EAE.

The investigation on neutrophil functional role in EAE development is our future endpoint.

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TLR2 stimulation promotes IL-17 production by human CD4+ CD25+ Tregs and reduces their suppressive function

Nyirenda Mukanthu H.*, Sanvito Lara, Constantinescu Cris S., Gran Bruno

University of Nottingham, Nottingham, United Kingdom

Naturally occurring CD4+CD25+FOXP3+ regulatory T cells (Tregs) are thought to suppress the activity of pathogenic T cells in autoimmune diseases such as multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system. Toll-like receptors (TLRs) are key components of the innate immune system which have been detected on CD4+C25+ Tregs. Recent data point to the direct or indirect involvement of TLRs in modulating the function of Tregs. We tested the hypothesis that TLR2 stimulation reduces the suppressive functions of human Tregs.

We show that TLR2 is preferentially expressed on human CD4+ CD25+ Tregs as compared to effector cells. We also show that the triacetylated lipopeptide TLR2 ligand Pam3CSK4, which requires the TLR1–TLR2 heterodimer for recognition, reduces the suppressive activity of human Tregs. In contrast, the suppressive functions of Tregs are not reduced by the diacylated TLR2 ligand FSL-1, which is recognised by the TLR2–TLR6 heterodimer. Preliminary data show that Pam3CSK4 enhances IL-6 secretion by Tregs and Tresp. In addition, stimulation by this agonist enhances TGF-β expression by Tregs, suggesting that TLR2 activation may promote Th17 cell differentiation.

These data support the hypothesis that in MS and potentially other immune-mediated diseases, infections may lead to exacerbation of disease activity by causing reduction of Treg suppression and differentiation of pathogenic Th17 cells.

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TLR7 and TLR9 stimulation reduce the severity of experimental autoimmune encephalomyelitis severity by expanding plasmacytoid dendritic cells in the central nervous system

O'Brien Kate[*,1], Fallarino Francesca[2], Gran Bruno[1]

[1]*University of Nottingham, Nottingham, United Kingdom*; [2]*University of Perugia, Perugia, Italy*

Experimental autoimmune encephalomyelitis (EAE) is a well-characterised model of cell-mediated autoimmunity in particular multiple sclerosis (MS). Toll-like receptors (TLRs) expressed on antigen presenting cells recognise microbial components and induce innate immune responses, leading to the elimination of invading infectious agents. Certain TLR agonists have been reported to have adjuvant properties in central nervous system (CNS) autoimmune inflammatory demyelination.

We report that TLR7 stimulation by Imiquimod, a synthetic analog of ssRNA, suppresses disease severity in a chronic murine EAE model. Preliminary studies have also found that stimulation with TLR9 agonist CpG type A also results in suppression of chronic EAE. In both cases disease suppression is associated with an expansion of plasmacytoid dendritic cells (pDCs) in the CNS as shown by expression of PDCA-1, B220 and CD11c. In TLR7 experiments we have found increased production of IFN-beta in spleens of mice treated with Imiquimod as measured by RT-PCR. We have also generated pDCs in vitro and found that Imiquimod induces the expansion of pDCs and also the production of IFN-beta. The expansion of pDCs in response to imiquimod is dependent on the type I interferon receptor (IFNAR) as mice lacking the receptor did not expand pDCs. In vivo depletion of pDCs has been found to reduce EAE severity. Interestingly, preliminary experiments have shown that Imiquimod exacerbates EAE in the absence of pDCs which indicates that the protective effect of Imiquimod is dependent on pDCs.

Our data demonstrate that TLR7 agonist Imiquimod and TLR9 agonist CpG type A reduce EAE disease severity and there is a potential for immunotherapy in MS by inducing the expansion of plasmacytoid dendritic cells which then produce IFN-beta which is currently used as a treatment of MS.

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Toll-like receptor 3 is pivotal for the development of TMEV-induced demyelinating disease

Duncan D'Anne*, Miller Stephen

Northwestern University, Feinberg School of Medicine, Department of Microbiology–Immunology, Chicago, IL, USA

Theiler's murine encephalomyelitis virus (TMEV), a single-stranded RNA virus, causes a chronic progressive CD4+ T cell mediated induced demyelinating disease (TMEV-IDD) in susceptible mouse strains, serving as a virally-induced murine model of the human disease multiple sclerosis. The innate immune system serves as the first line of host defense against pathogens such as viruses. Toll-like receptors (TLRs), members of the pattern recognition receptor (PRR) family, recognize pathogen associated molecular patterns (PAMPs), and ligand binding activates pro-inflammatory and antiviral responses on APCs. Central nervous system (CNS)-resident cells, microglia isolated from human and neonatal murine tissue, express multiple TLRs in vitro and in situ. Therefore, we examined TLR-stimulated microglia and macrophage immune functions in the murine CNS in response to TMEV infection.

Following TMEV infection, TLR2, TLR3, TLR7, TLR8, and TLR9 expression are significantly upregulated at day 7 post-infection (p.i.), suggesting that TLRs are dynamically regulated during acute Theiler's virus infection. Using quantitative real-time PCR, TLR3 expression (which recognizes viral double-stranded RNA) is highly inducible on FACS-sorted microglia (CD11b+CD45LO) in response to TMEV-infection at day 7 p.i. These data suggest that viruses may selectively upregulate expression of TLR3 for mounting antiviral responses, which is necessary for TMEV viral clearance in the CNS. To further elucidate the role of TLR3 in TMEV-IDD, we examined the development of induced demyelinating disease in its absence (TLR3−/− SJL). Compared to wild-type SJL mice, TLR3−/− SJL mice are hypersusceptible to disease and exhibit increased demyelination, suggesting that TLR3 is protective against TMEV-IDD. Additionally, TLR3 SJL mice are defective in TMEV clearance in the CNS, ured by increased viral titers in TLR3−/− mice cor pe controls. TLR3−/− SJL mice are deficient numbers at day 7 p.i., which is also ref CD8+ T cell percentage and cell num TMEV clearance.

In the future, experiments will be f role of TLR3 on the effector functior acute infection. This study was s 1F31NS061621-01A1.

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