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MICE DEFICIENT IN P52/NF-κB AS A MODEL FOR STUDYING PRION DISEASE

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Abstract

A tight functional link between the central nervous and peripheral immune system closely related to the activity of NF-κB transcription factor has been evidenced in numerous brain pathological conditions. One of the most instructive examples is a prion neuroinvasion and the development of transmissible spongioform encephalopathy (TSE) or prion diseases. The failure of peripherally administrated prions to elicit disease in immune-deficient mice indicates that preserved organ microarchitecture is crucial for TSE pathogenesis and it seems to go through topographic relationships between follicular dendritic cells and sympathetic endings in lymphoid organs. New insight into the molecular requirements for follicular dendritic cell (FDC) development in NF-κB p52 deficient mice highlights the role of NF-κB transcription factor in prion disease progression. However, adoptive transfer of wild type bone marrow cells (NF-κB +/+ background) into p52 deficient animals (NF-κB -/- background) does not correct the FDC defect. RT-PCR analysis of the expression level of mRNA for LTα, LTβ, TNFRI and BLC of the splenic cells from p52/NF-κB mutant/wt BM chimera did not reveal major changes compared to wt/wt BM chimeras, indicating that their normal expression within splenic tissue does not assure for complete restoration of the FDC network in p52/NF-κB mutant. This implies that the distance between FDCs and splenic nerve terminals around the central arteriole is longer and could interfere with prion spreading in lymphoid organs prior to neuroinvasion. In light of the fact that prion neuroinvasion highly depends on the physical distance between sympathetic nerve endings and FDCs dendritic extensions, it is not hard to imagine that this could be the way to approach the problem of prion diseases development.

Keywords

• p52 knockout mice • Prion disease • Follicular dendritic cell development

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1. Introduction

There is an increasing amount of experimental data illustrating a tight functional link between the central nervous and peripheral immune system supporting the old Descartes' concept of dualism in the form of mind and body interconnections. Bidirectional neuro-immunological talk has been evidenced in different pathological conditions ranging from peripheral immunosuppression induced by stress, or brain damage [1-3]. A molecule which perfectly suits the role of surveillance factor in order to assure the coordinate purposeful activity of both partners in this neuro-immunological liaison is NF-κB transcription factor. It has been proven, in numerous ways, that the final outcome of NF-κB activation depends on the activated cell types which basically reflects the composition of the complexes forming the transcriptionally active form of NF-κB [4,5]. IKKα activated release of RelB/p52 dimers is proven to be required for secondary lymphoid organogenesis and induction of various genes involved in this process [5]. The overlapping pattern of these genes maintains the cytokine milieu of the brain microenvironment to sustain the immature state of resident brain microglia which essentially represents the first line of defense against „ danger signals" that could be either inflammatory or due to degenerative changes affecting the brain region. Self-expansion capacity ascribed to the immature state of these cells basically represents a double-edged sword since it could be a generator of uncontrolled astrogliogenic reactions as part of a regular response of brain tissue to injury. In that sense, it is important to point out that NF-κB related Jak-1/Smads signaling dependent expression of genes associated with brain repair reactions could be envisaged as a mode to keep microglial self-expansion potential under control in different pathological conditions of the brain [6].

2. Experimental Procedures

2.1 Animals and immunization protocols

Mice deficient in p52/NF-κB were generated by targeted disruption in embryonic stem cells as previously described [7].

2.2 Adoptive bone marrow transfers

Bone marrow cells isolated from the femora of 6 week old p52/NF-κB mice were injected ($3x10^6$ cells per mouse) into either p52 deficient mice or control littermates (6-18 weeks old). Seven to eight weeks after the transfer, mice were injected i.p. with sheep red blood cells (SRBC) and 9 days later the mice were sacrified and spleen samples were taken for RT-PCR analysis.

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2.3 Intracellular labeling of p52 on follicular dendritic cells preparation

Preparation of follicular dendritic cells (FDCs) and the staining for p52 protein was accomplished as previously described [7].

2.4 RT-PCR analysis

RNA from splenocytes was isolated using Trizol reagent (Life Technologies, Grand Island,NY). cDNA were synthesized using the Clontech (Palo Alto, CA) PCR kit starting with the same quantity of RNA (1μg). The following primers were used: 5'TCA CCT AGG ATG AGG CTC AGC ACA GCA and 3' CAC TCA TTC TCT TCT CGA CGG GAA TTC CAC for BCL (amplified fragment 364 bp), 5' GGT GAA GGT CGG TGT GAA CGG A and 3' TGT TGA TGG GGT CTC GCT CCT G for GAPDH (amplified fragment 383 bp), 5' TGG TGA CCC TGT TGT TGG CAG T and 5' AGC TCA GGG TTG AGG TCA GTT for LTβ (amplified fragment 459 bp), 5' TGC CAC TGC TCG GCC GTC TCC A and 3' GTT GCT CAA AGA GAA GCC ATG TCG for LTα (amplified fragment 278 bp), 5' CAT CCA CCA CAG CAT ACA GAA TCG CAA GG 3' for TNF-RI (amplified fragment 351 bp).

3.Results and discussion

As we previously reported, follicular dendritic cells (FDCs) (a major source of disease-associated prion proteins) are totally absent from p52-deficient animals [7]. This may not be so surprising since these cells, as we show here, express high p52 levels in the form of strong, uniform intracytoplasmic staining within FDCs clusters when stained with anti-p52 antibody (Figure 1A).

According to our previous data, adoptive transfer of wild type bone marrow cells (NF-κB +/+ background) into p52 deficient animals (NF-κB -/- background) did not correct the FDC defect, yet some FDC-M2 positive cells were observed [7]. The distance between the central arteriole and FDCM-2$^+$ cell clusters is significantly larger in p52KO/ wt chimera than in control mice (Figure 1B and C). This further indicates that the distance between FDCM-2$^+$ cells and splenic nerve terminals in the T cell zone around the central arteriole is longer in p52KO/ wt bone marrow chimera

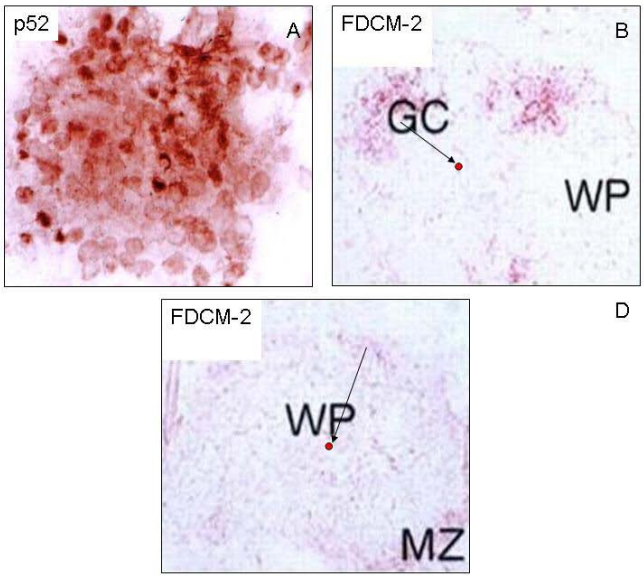

Figure 1. A uniform, positive intracellular reaction for p52/NF-κB obtained by anti-p52 specific antibody indicating high p52 content in FDCs (A);Distance (black arrow) between central arteriole (red point) and FDCM-2$^+$ cluster is significantly shorter in control wt/wt BM chimera (B) than in p52KO/wt chimera (C). WP white pulpe, MZ marginal zone, GC germinal center.

than in wild/wild type BM chimera. In germinal centers follicular dendritic cells are believed to be the main cell type for efficient prion replication in the periphery prior to neuroinvasion [8]. Since the physical distance between splenic nerve terminals around the central arteriole and clusters of FDCM-2 positive cells is longer in p52KO/wt chimera it is easy to imagine that prions spreading along neural fibers may not occur or will occur less efficiently. When we performed more detailed RT-PCR analysis of the splenic cell population from p52/NF-κB mutant/wild type bone marrow (BM) chimera, as well as from control wild type/wild type BM chimera, no major changes in the expression level of mRNA for LTα, LTβ,TNFRI and BLC were observed, suggesting that proper expression of these molecules within splenic tissue does not assure for complete restoration of the FDC network in p52/NF-κB mutant (Figure 2). Given the fact that LTα -/-, LTβ-/-, LTβR -/- and TNFα -/- mice resisted infection with prions being administered intraperitoneally, and the fact that these mice exhibit impaired microarchitecture of secondary lymphoid organs to different extents, we assume that

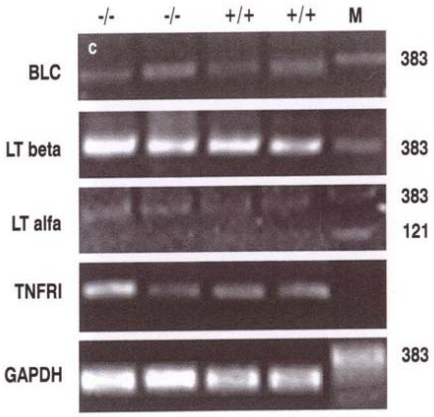

Figure 2. RT-PCR analysis of the TNF-RI, LTα, LTβ and BLC expression level in unpurified splenic cell population, either from p52/NF-κB mutant or wild type recipient after the transfer of wild type bone marrow cells (n=4/group/experiments). Representative data of two independent experiments are shown. No difference in the expression level of BLC, LTα, LTβ and TNFRI between p52/NF-κB mutant/Wt bone marrow chimera and control animals was observed.

p52 KO mice would be a good model to make the distinction between lymphotoxin/ tumor necrosis factors (LT/TNF) dependent effects on prion disaese development, which are not necessarily related to proper development of

the microarchitecture of secondary lymphoid organs [9].

Although FDCs have been considered as the main cell type in the body's periphery required for efficient prion replication, it has been found that, in the absence of mature FDCs, the PrP signal colocalized with a subset of signal metalophilic and marginal zone macrophages in TNFRI -/- lymph nodes [10]. It is interesting that in p52KO mice adoptively transferred with wild type bone marrow cells the appearance of MOMA-1$^+$ cells can be easily visualized [7]. How their presence can be correlated with prion disease development in p52 KO mice remains to be elucidated.

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