Identification of Novel Candidate Antibody Biomarkers in Spinal Cord Injury

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INTRODUCTION

Recent studies highlight the pivotal role of B cells and their antibodies in spinal cord injury (SCI)-induced neuroinflammation. Animal models showed that B cells are affected and central nervous system (CNS)-reactive serum antibodies are increased after SCI (1). Furthermore, both B cells and antibodies clearly contribute to aggravated tissue damage and impaired neurological recovery after SCI (1). However, the role of B cells and antibodies after SCI in humans is still poorly understood. Moreover, there is a vast need for early and reliable biomarkers for SCI diagnosis and prognosis as the current tools have only limited capacity (2). Besides the potential pathophysiological role, SCI-induced antibodies may also function as clinically relevant biomarkers. Therefore, a comprehensive analysis of patient samples is needed to establish the true prevalence, specificity and pathogenic relevance of SCI-induced antibodies in humans. In this study, the antibody reactivity profile after SCI is determined using serological antigen selection (SAS) to identify antibody biomarkers for SCI diagnosis and prognosis.

Keywords: spinal cord injury, cDNA phage display library, serological antigen selection and antibody biomarkers

MATERIALS AND METHODS

SAS is used to identify novel candidate antibody biomarkers and involves the display of a cDNA library as a fusion protein with filamentous phage protein, pVI. Spinal cord cDNA inserts from healthy individuals (n=18) were cloned into phagemid vectors (pSPVI-A-B-C) (3). The resulting spinal cord cDNA library was affinity selected with pooled plasma from traumatic SCI patients (n=10) to enrich for antigens recognized by SCI-induced antibodies (4). The plasma pool consisted of 10 samples from SCI patients with a different type of injury, age and gender to limit patient-specific immunoreactivity. After 5 selection rounds, the output was analyzed via titration, PCR and fingerprinting. Antibody reactivity towards selected phage clones was tested in SCI patients and healthy controls using phage ELISA. Plasma samples from SCI patients and healthy controls were collected and stored via University Biobank Limburg and Biobank UZ Leuven.
RESULTS
To investigate the antibody reactivity profile after SCI, a human spinal cord cDNA phage display library with a diversity of $10^6$ primary recombinants was constructed and affinity selected with pooled plasma from traumatic SCI patients. After the SAS procedure, an increased output/input ratio was obtained which reflects the enrichment of specific phage clones. Subsequently, random phage clones were analyzed using PCR and fingerprinting. This resulted in 27 putative enriched phage clones which were studied further for antibody reactivity using phage ELISA. Our preliminary results demonstrate that SCI patients show increased antibody reactivity towards selected phage clones compared to healthy controls.

CONCLUSION
SAS is an efficient procedure for molecular profiling of the antibody response after SCI. By performing SAS, 27 putative enriched phage clones were identified with increased antibody reactivity in plasma from SCI patients. These results clearly highlight the potential contribution of antibodies in SCI pathology and underline the need for research on B cells and antibodies in SCI. In the future, sequencing will be used to confirm the enrichment of the selected phage clones and to determine the identity of the antibody targets.

REFERENCES