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ELISPOT assays and their diagnostic potential in Lyme disease and Lyme neuroborreliosis

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Dear Editor,

We have read the research article entitled 'Prospective comparison of two enzyme-linked immunosorbent spot assays for the diagnosis of *Lyme neuroborreliosis*' by van Gorkom T *et al.*, published in *Clinical and Experimental Immunology* [1]. We want to congratulate the authors for the publishing of this article, and make some remarks and contributions.

In the article mentioned above, two assay systems for detection of reactive T-cells against a certain stimulus, in their case *Borrelia burgdorferi* sensu lato (*B. burgdorferi*), respectively the determination of T-cells from suspected neuroborreliosis patients were compared.

The positive antibody index (AI) for the detection of intrathecal *B. burgdorferi*-specific antibodies is, at the moment, the only available *Borrelia*-specific laboratory diagnostic procedure for neuroborreliosis determination according to the guidelines by the European Federation of Neurological Societies [2]. However, this intrathecal antibody production can be false-negative in 21–45% of all patients, or remains positive for long time periods after antibiotic treatment [3,4]. Approaches for overcoming these problems are for example measuring intrathecal levels of CXCL13 as potential biomarkers [5]. The idea of using a well-defined, established T-cell based system like the LymeSpot assay for determining neuroborreliosis patients would be a precious contribution to the current diagnostic situation.

As manufacturer of one of the tested systems, we stayed in close contact with the first author and have had some interesting discussions about the study in the past. Based on discussions on a highly scientific level, as well as collegial and cooperative interaction, we want to state our point of view regarding the authors' claim that: 'Both the in-house and the LymeSpot assay are unable to diagnose active *Lyme neuroborreliosis* (LNB) nor to monitor antibiotic treatment success' in this letter.

One key point of the LymeSpot assay is, that the intended use is not for the diagnosis of neuroborreliosis in patients, as this disease is immunological completely different to other *Borrelia*-caused disease patterns like Lyme arthritis or Lyme borreliosis (LB). In this letter, we would like to give a short, but more differentiated explanation on the kit and the intended use.

The ELISPOT detects the number of antigen-specific T-cells based on their cytokine production and measures cell-mediated immune responses: *in-vitro* confrontation of lymphocytes with specific antigens (e.g. from a certain pathogen) [6].

A significant T-cell reactivity upon stimulation with *Borrelia* proteins is well-known and well-proven; it correlates with the clinical pattern and symptoms [7,8].

As of right now, the EliSpot technique is the gold standard [9] for different applications in the field of infectious diseases, for autoimmunology, for transplantation diagnostics [10,11], allergy (therapy monitoring) [12] and for vaccination studies.

LB, which was first described in the 1970s, represents the most frequent vector-borne disease in many European countries and in the United States [13,14]. *B. burgdorferi*, the causative agent of LB belongs to the family of Spirochaetaceae. Comparable with other Spirochaetaceae infections, LB occurs in three stages, early localized, the disseminated and the late stage. A broad and wide range of clinical symptoms and incubation times mark the three stages of LB. Each stage can be skipped and self-limitation in each stage is possible. The incubation period differs from 3 to 32 days in the early stage of LB, to several weeks or months in a late stage or stage of manifestation. According to this, the diagnosis/detection of LB is delayed [15].

While a protective effect of specific antibodies against *B. burgdorferi* was shown in literature, the systemic manifestation of LB has been reported in spite of high antibody titers. These argue for the role of cell-mediated immunity in the processing of LB [16–18].

As mentioned, the LymeSpot, and therefore the detection of *Borrelia*-specific T-cell reaction, could be helpful with unclear serological results, unclear (remaining) clinical symptoms, evaluation of therapy (especially in late stage) and therapy monitoring, as indicated in the intended use [18,19].

With the EliSpot (iSpot), 235 patients suspected for or with reported tick bites in the past were tested for reactive T-cells after stimulation with *Borrelia* antigens (**AID GmbH ELSP5905 *Borrelia* B31 lysate**; concentration: 5 µg/ml (ready to use); purified bacterial lysate from *B. burgdorferi* strain B31; and **ELSP5946 *Borrelia* Osp-Mix**; concentration: 5 µg/ml in AIM-V (ready to use); pool of 9 mer – 11 mer peptides from OspA (*B. burgdorferi*, *B. afzelii*, *B. garinii*), native OspC (*B. afzelii*) and recombinant p18).

Of these 235 cases, 30 samples were tested as positive (SI ≥ 3) and 18 as borderline (SI 2–3). This corresponds to a prevalence of 20, 4% within the tested patient cohort. Bases for the test were the above mentioned rules for EliSpot evaluation (negative control ≤ 10 spots, positive control ≥ 50 spots) and the AID developed scheme for LymeSpot interpretation (EliSpot with *Borrelia* B31 and OspMix antigens). The scheme is included in the kit and antigen package insert.

From our point of view, an interferon (IFN)-y ELISPOT as only cytokine used in the study from van Gorkom *et al.*, with *Borrelia*-specific antigen stimulation of peripheral blood cells is not sufficient to describe a complex immunopattern like in neuroborreliosis, compared to *Borrelia* patients with other clinical appearance. As the authors describe, spot numbers increase when testing CSF, which makes much more sense in case of neuroborreliosis compared to testing the peripheral blood cells, as 'the accentuated response in the CSF reflects an autonomy of the intrathecal *B. burgdorferi*-specific T-cell response'[8].

In the 'EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis' [2], for the diagnosis and confirmation of neuroborreliosis, the determination of an intrathecal *Borrelia* antibody titer is crucial, as serum antibody titers are not necessarily correspond with the disease state. The function of the blood–brain barrier together with a seroprevalence ranging from 4 - 21% in the normal healthy population underlines the importance of specialized diagnostics, for example T-cell reactivity in CSF in suspected neuroborreliosis and not from sera.

Furthermore, it has been shown for other CNS infections such as VZV, HSV-1 and HSV-2 that stimulation of liquor cells (or cells from other extra sanguine fluids) alone has a limited significance as diagnostic tool due to the limited number of antigen-presenting cells and might lead to false-negative results; therefore, the specific immune cells need co-stimulation from blood cells to detect specific T-cell immune answers [20]. Beside this approach, combination of different cytokines beside IFN-y could elucidate T-cell reactivity in cases of neuroborreliosis patients from sera, which is a current focus in a research project with the University of the Saarland.

As the authors state that

> 'not much is known about the T-cell dynamics after treatment and controversial data have been published regarding this subject. Therefore, this needs to be further elucidated, and we are currently following the active Lyme neuroborreliosis patients both serologically and immunologically (through *Borrelia* EliSpot) at different time-points up to two years after inclusion'.

The study population of active LNB included in the study by Gorkom *et al.* [1] included 6 out of 18 patients with suspected LNB because of the lack of intrathecal antibodies against *Borrelia.* The interesting study population in this case would be the correlation of the remaining twelve patients with definite active LNB and the EliSpot result, which was missing in the results and discussion part, at least their Table 5b, which is not included but shortly mentioned in the manuscript.

We would like to point out that this approach for monitoring is exactly the kind of data which is needed but still missing in the most clinical trials regarding *Borrelia* patients. Only with characterized groups, follow ups for longer time periods and the use of different diagnostic methods it will be possible to improve *Borrelia* diagnostics and gain a clearer understanding of individual immune reactions to this spirochete.

"One approach for example is an ongoing clinical study, the VICTORY project. VICTORY is a prospective two-gate case–control study. We strive to include 150 patients who meet the European case definitions for either localized or disseminated LB. In addition, we aim to include 225 healthy controls without current LB and 60 controls with potentially cross-reactive conditions. We will perform four different cellular tests in all of these participants, which will allow us to determine sensitivity and specificity. In LB patients, we will repeat cellular tests at 6 and 12 weeks after start of antibiotic treatment to assess the usefulness as 'test-of-cure'. Furthermore, we will investigate the performance of the

different cellular tests in a cohort of patients with persistent symptoms attributed to LB" [21].

We would also appreciate such well-considered studies for patient groups to clarify the diagnostic potential and usefulness of cell-based test systems adjusted to a highly challenging immunological subject as the neuroborreliosis.

Disclosures

N. E. S. acts as a paid employee at GenID GmbH and as paid consultant to AID GmbH. R. P. acts as a paid employee at GenID GmbH and as a paid consultant to AID GmbH.

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