SHORT REPORT

Interferon-γ-activated calcium influx in peripheral blood lymphocytes from patients with primary and secondary progressive multiple sclerosis

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Abstract

Interferon-γ (IFN-γ) contributes to the early events leading to T cell cell in relapsing-remitting (RR) multiple sclerosis (MS) by activating a transplasmalemma calcium influx, the detection of which is closely associated with clinical and MRI evidence of disease activity. The appearance of this influx represents one of the earliest peripheral events in the pathogenesis of RRMS. It is still questioned whether the same immune mediated mechanisms also operate in primary progressive (PP)MS. Fluorimetric evidence of the IFN-γ-activated calcium influx was sought in 16 patients with PPMS and 39 patients with secondary progressive (SP)MS. To compare peripheral versus CNS evidence of immune activation 11 of the patients with PPMS and 27 of the patients with SPMS underwent gadolinium enhanced brain MRI. The IFN-γ-activated influx was detected in peripheral blood lymphocytes from eight of 16 (50%) patients with PPMS, and 20 of 39 (51%) patients with SPMS, a frequency similar to that previously reported in patients with RRMS during phases of disease stability. Gadolinium enhancing brain MRI lesions were found in only one of 11 (9%) patients with PPMS and 12 of 27 (41%) with SPMS. Our study shows that peripheral blood lymphocytes from patients with PPMS and patients with SPMS express with the same frequency as patients with RRMS, an IFN-γ-dependent intracellular process leading to T cell activation able to trigger disease activity.

Keywords: primary progressive multiple sclerosis; T lymphocytes; interferon-γ; calcium

Activated T lymphocytes are involved in the formation of CNS demyelinating lesions in patients with multiple sclerosis (MS). Their activity is orchestrated by a complex network of cytokines among which interferon-γ (IFN-γ) is considered essential. We have recently characterised a transplasmalemma calcium influx induced by IFN-γ which mediates part of its effects on lymphocytes from patients with relapsing-remitting (RR)MS. As a result of the activity of this influx, T lymphocytes, in fact, show a lower threshold of immunological excitability, leading to a state of preactivation. Moreover, the detection of the influx significantly correlates with the occurrence of new brain MRI enhancing lesions and clinical relapses in patients with RRMS, thus supporting its important part in the peripheral activation of T lymphocytes that precedes demyelination in patients with MS.

Primary progressive (PP)MS affects 10% to 15% of patients with MS and is perceived as a distinct pathological entity lacking some of the immune mediated features of the more frequent RR form. Recent pathological and MRI evidence has, however, shown inflammatory lesions within the CNS of patients with PPMS similar, although less intense, than those of patients with RRMS or secondary progressive (SP)MS.

To assess whether evidence of peripheral immune activation, often found in RRMS, is also present in the PP form of the disease, we assessed the frequency of IFN-γ-activated calcium influx positive T lymphocytes in patients with PPMS and, as a control, in patients with SPMS. Evidence of CNS immune activation was sought by gadolinium enhanced brain MRI in some of these patients.

Patients and materials

Peripheral blood lymphocytes were obtained from 55 patients diagnosed as affected by clinically definite MS. The clinical course of the disease was PP in 16 patients and SP in 39 patients. The table summarises the clinical data. At the time of sample collection, all patients with MS underwent clinical examination and were evaluated by the expanded disability status scale (EDSS). PPMS was defined as a clinical syndrome that was progressive from the onset, with no evidence of relapses or remissions. Patients with SPMS had an initial relapsing-remitting phase, which later evolved into a progressively disabling disease, with or without superimposed relapses, over at least the preceding six months.

Human peripheral blood lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation from all patients and controls.
Adherent cells were discarded to eliminate macrophages. To evaluate the presence of the IFN-\(\gamma\) activated calcium influx, fluorimetric analysis was performed according to the calcium-free-calcium reintroduction protocol, as previously described.\(^5\) We considered only consistently measurable intracellular calcium rises over basal concentrations occurring in cells only exposed to IFN-\(\gamma\). Positivity was expressed as the percentage of the intracellular calcium increase recorded in response to IFN-\(\gamma\) subtracted from the intracellular calcium increase in the absence of any stimulus (already subtracted were the small cytosolic calcium changes occurring as a consequence of the protocol used).\(^6\) A patient was considered influx positive only when the difference of increase in intracellular calcium was higher than 1% in at least two of three experiments performed in parallel (no stimulus \(v\) IFN-\(\gamma\)) for each patient’s sample.

Eleven patients with PPMS and 27 with SPMS were also studied by gadolinium enhanced brain MRI. This was performed with a 1.5 Tesla machine (Siemens SP63, Erlangen, Germany) immediately after blood collection in all patients. Five mm contiguous axial T1 weighted (SE 600/17) slices through the whole brain were obtained five to seven minutes after the injection of gadolinium-diaminediethylene-triamine penta-acetic acid (DTPA; 0.1 mmol/kg intravenously over one to two minutes). Enhancing lesions were counted on each scan by one of us (MF) unaware of the clinical status of the patients and of the fluorimetric analysis results. Statistical analyses were performed with the the Mann-Whitney \(U\) test and the \(\chi^2\) test.

### Results

The table summarises the results for the IFN-\(\gamma\) activated calcium influx. The influx was found in eight of 16 (50%) patients with PPMS and in 20 of 39 (51%) patients with SPMS and there was a mean (SD) percentage increase of intracellular calcium over a basal concentration of 14.8 (5.2)% in patients with PPMS and of 15.3 (9.0)% in patients with SPMS. No significant differences in age, sex, duration of disease, and EDSS score were found between influx positive and influx negative patients with PPMS or SPMS. However, influx positive patients with PPMS tended to have a longer duration of disease and a higher EDSS score than influx negative patients with PPMS (table). gadolinium enhancing brain MRI lesions were found in only one of the 11 (9%) patients with PPMS and in 11 of 27 (41%) patients with SPMS. In the PPMS group the only gadolinium enhancing brain MRI positive patient was also influx positive. In the SPMS group, enhancing lesions were detected in eight of 16 influx negative and three of 11 influx positive patients. The differences in the detection of gadolinium enhancing brain MRI lesions between influx positive and influx negative patients with PPMS or SPMS were not significant.

The IFN-\(\gamma\) activated calcium influx was more often found in PP/SPMS groups than conventional dose enhancing lesions (PPMS, 50% \(v\) 9%; SPMS, 51% \(v\) 41%). Interestingly, in one influx positive patient with SPMS with no enhancing lesions after a standard dose (0.1 mmol/kg) of gadolinium, two lesions were visualised after a 0.3 mmol/kg dose of gadolinium.

### Discussion

The uneventful progression of neurological impairment, the relatively infrequent MRI detection of CNS lesions, distinguishable immunological profiles, and the divergent immunogenetic background of patients with PPMS compared with those with RRMS have supported the perception that the pathogenetic mechanisms differ among the two groups.\(^5\) Recent neuropathological studies, however, have shown that pathological changes seen in the brains of patients with RRMS (perivascular cuffs and lymphomononuclear cells infiltrating brain and spinal cord parenchyma) are actually present in the CNS from patients with PPMS, and do not differ qualitatively from those seen in the RR form.\(^9\) Additional evidence came from a recent study showing that brain MRI enhancing lesions are present in about 50% of patients with PPMS when a triple dose of...
gadolinium-DTPA is used. The combined evidence suggests that a quantitative, rather than a qualitative, difference underlies the occurrence of one or the other forms of MS.

The expression of the transplasmalemma IFN-γ-activated calcium influx makes circulating T lymphocytes more likely to proliferate by lowering their threshold of activation. The influx, which we recently found to be present also in healthy subjects but operating preferentially in patients with MS in whom it can be readily activated in an antigen-independent manner by an MS-specific cytokine milieu (unpublished data), provides a relevant quota of calcium necessary to transduce activatory signals from the lymphocyte surface to the nucleus, usually provided by the T cell receptor induced and inositol trisphosphate mediated intracellular signalling pathway. Peripheral T lymphocyte activation is actually one of the earliest events in the immune mediated process leading to inflammation and demyelination occurring in MS. As the influx is upregulated in lymphocytes from patients with RRMS four to six weeks before the occurrence of a clinical attack or gadolinium enhancing brain MRI lesions, it might be considered as an early marker of peripheral immune activation. We then used influx detection to signal peripheral immune activation in patients with PPMS or SPMS, and compared the obtained frequency to that previously reported in patients with RRMS.

We found the IFN-γ activated influx in T lymphocytes from half of the patients with PPMS or SPMS. This frequency was lower than that recorded in the first weeks after the onset of a clinical relapse in patients with RRMS (78%), but similar to that of patients with RRMS during phases of disease stability (45%). We speculate that lymphocytes from patients with RRMS or PPMS have a common ability to be activated by IFN-γ, irrespective of their disease course. Cells with this IFN dependent calcium influx, however, seem to be expanded in patients undergoing massive immunooactivation leading to acute focal inflammation and demyelination. We are currently unable to determine what prevents the "critical" usage of this activation pathway in patients with PPMS or SPMS, or if its down regulation is a primary (causative) or secondary (the result of a blunted immune system) event.

We then validated the IFN-γ activated calcium influx as a marker of disease activity by comparing its detection with the appearance of gadolinium enhancing brain MRI lesions and found that the use of conventional (0.1 mmol/kg) gadolinium enhancement discloses a lower percentage of PPMS with evidence of CNS inflammation compared with percentage of influx positivity (9% vs 50%). One possible explanation for this discrepancy is that the two variables (influx and MRI) operate in different temporal and spatial activity phases of the disease, thus making their comparison difficult. On the other hand, it is possible that conventional gadolinium enhanced MRI is suboptimal in disclosing the less intense inflammatory lesions occurring in patients with PPMS. It is interesting to note that the frequency of influx positivity in patients with PPMS is similar to MRI positivity recorded in patients with PPMS when a triple dose of gadolinium is used. Anecdotally, one of our influx positive, conventional MRI negative patients with PPMS showed two enhancing lesions when a higher dose of gadolinium was used.

In conclusion, these findings again support the view that peripheral immune activation, possibly leading to CNS inflammation, occurs in non-RRMS patients as often as in patients with RRMS, only with a lower intensity. This finding, although inferential, provides additional evidence for a common immunopathological background underlying the two disease courses. Further efforts should be devoted to the identification of the modifying factor(s) leading to differential expression of immune mediated responses in these patients.

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