Spontaneous and mutagen induced sister chromatid exchange in multiple sclerosis

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SUMMARY Spontaneous and mutagen induced sister chromatid exchange (SCE) frequencies have been studied in nine patients with multiple sclerosis and in nine age and sex matched healthy controls. The incidence of spontaneous SCE in lymphocytes of the MS patients was significantly greater, by about 50%, than in those of the control subjects. When exposed to mitomycin C (MMC) or ethyl methane sulfonate (EMS) in vitro, cells from both groups showed typical dose dependent increases in SCE frequency, with yields from MS patients slightly higher than from controls. The higher SCE yields in mutagen treated MS cells relative to controls is considered to reflect initial basal differences between the cell types, so that MS cells are not intrinsically hypersensitive to mutagen treatment.

Multiple sclerosis (MS) is a common disease of the nervous system affecting about 50,000 people in the United Kingdom. The highest prevalence of MS in the world is in the Orkney and Shetland Isles. The onset of the disease is usually between 15 and 50 years of age and the incidence in females outnumber that in males by 1.4:1. The disease has been recognised for the past century but the exact cause is still unknown. Investigations on histocompatibility antigens and family studies showing that about 10% of patients with MS have an affected relative reveal an influence of genetic factors associated with susceptibility to the disease, and epidemiological studies point to the possible role of viral infection. Many other correlations implicating diet and geoclimatic and socioeconomic variables have also been reported. Recent studies indicate abnormalities in circulating immunoregulatory cells correlating very well with disease activity, and Waksman has postulated that MS occurs in genetically predisposed persons manifesting defects in immune regulation, triggered by viral infection or autoimmunity in nature. Early neuronal degeneration associated with impaired immune response is characteristic of a number of inherited diseases in man including ataxia telangiectasia (AT). AT cells also show enhanced response to cell killing and production of chromosomal aberrations following exposure to a variety of mutagens, suggesting a defect in the repair of damaged DNA. A similar mutagen hypersensitivity has been well established in xeroderma pigmentosum (XP), another autosomal recessive disease associated with neurological abnormalities and defects in DNA repair. These observations have led to the proposition that DNA repair is required for the functional integrity of the nervous system and the premature death of neurones damaged in vivo by endogenous chemicals might be the consequence of inefficient DNA repair. Such a sequence of events has been postulated in other degenerative diseases of the nervous system, in addition to AT and XP. Two recent reports have indicated a raised incidence of spontaneous sister chromatid exchange (SCE) and of x-ray induced chromosomal aberrations in the lymphocytes of MS patients, pointing to the possibility of an inherited abnormality in DNA repair in this condition. We have examined this possibility and in the present paper report on the incidence of spontaneous and mutagen induced SCE in the lymphocytes of nine MS patients and matched controls and conclude that MS is not associated with mutagen hypersensitivity.

Materials and methods

The nine patients studied, four males and five females, were unrelated and their ages ranged from 25 to 56 years (mean 41 years). All of the patients satisfied the criteria described by McDonald and...
Halliday\textsuperscript{19} of clinically definite multiple sclerosis. Symptoms had been present for from 3 to 27 years (mean 10 years) at the time of blood sampling, two of the patients had been treated with antibiotics and analgesics not known to cause chromosome damage, and one had completed a three day course on dexamethasone just before study. Heparinised whole blood was collected from the nine patients and from an equal number of age and sex matched healthy controls. From each subject, separate cultures were set up each using 0.8 ml blood in 10 ml RPMI 1640 medium containing 20% fetal calf serum, 1% PHA (Wellcome), 1% glutamine, 100 U penicillin, 100 \( \mu \)g streptomycin, and 25 \( \mu \)mol/l BrdU. Mitomycin C (MMC, Kyowa, Japan) dissolved in distilled water was immediately added to one set of cultures to give final concentrations of \( 10^{-8}, 3 \times 10^{-8}, \) and \( 10^{-7} \) mol/l. Ethylmethanesulfonate (EMS, Koch-Light Laboratories) dissolved in distilled water was added to a second set of cultures to give final concentrations of \( 10^{-4}, 3 \times 10^{-4}, \) and \( 10^{-3} \) mol/l. All cultures were kept in the dark at 37°C for 72 hours and were treated with \( 5 \times 10^{-7} \) mol/l colchicine for the last 2 hours. The cells were then collected, treated with 75 mmol/l potassium chloride for 8 minutes, fixed in 3:1 methanol acetic acid mixture, and dropped onto slides which were air dried. The fluorescence plus Giemsa technique\textsuperscript{20} was used for differential chromatin staining and all slides were randomised, coded, and scored blind. From each culture, 20 metaphases showing typical harlequin staining were examined to record the frequency of SCE.

Results and discussion

The spontaneous incidence of SCE in the cells from nine MS patients (11.8 \( \pm \)0.43) is significantly higher (\( p<0.001 \)) than in those of the nine healthy controls (7.2 \( \pm \)0.34, fig 1), a result which confirms and extends the initial findings of Sutherland et al.\textsuperscript{17} The proportions of cells showing different numbers of SCE are shown in fig 2 which again demonstrates a clear difference in the distributions between the groups.

There is disagreement on the question of whether PHA responsiveness is depressed in blood lymphocytes from MS patients, particularly during the active phase of the disease,\textsuperscript{21-25} and there is evidence that the rate of cell proliferation has an effect on both spontaneous and mutagen induced SCE frequencies.\textsuperscript{26-27} In our studies we found no evidence for an abnormal response of MS lymphocytes to PHA stimulation. The use of BrdU and FPG staining provides an unambiguous identification of cells in their first, second, and third or later division in 72 hour culture, and the proportions of dividing cells at these divisions provides a measure of

![Graph](http://jmg.bmj.com/)

**Fig 1** Individual mean SCE frequency (\( \pm \) SE scored from 20 second division metaphases) in nine controls (\( \bullet \)) and nine MS patients (\( \blacksquare \)).

![Graph](http://jmg.bmj.com/)

**Fig 2** Percentage of cells showing different number of SCE from nine controls and nine MS patients. A total of 180 cells was examined in each group.
proliferation rate. In 200 mitoses counted from each subject, the respective mean indices found were 22%, 42%, and 36% in controls and 18%, 48%, and 34% in MS patients. These results thus suggest that, relative to controls, neither the response to PHA nor the rate of cell proliferation is altered in MS patients and the observed difference in their spontaneous SCE frequency is not the result of either of these parameters. The possibility that the drugs taken by the patients could be responsible for increasing the SCE must be considered, but so far none of these drugs has been shown to have adverse cytogenetic effects.

Whether the increase in SCE frequency in MS patients might be the result of the other factors associated with the disease is worth consideration. Epidemiological studies provide strong support for the hypothesis that MS is initiated by virus infection and evidence has been advanced to incriminate various types of virus infection as causal factors in MS. Viral infection, both in vivo and in vitro, is well known to be associated with the induction of chromosomal aberrations, but there is no indication of an increased chromosomal aberration frequency in blood lymphocytes of MS patients. Our own unpublished results on the lymphocytes from 50 subjects immunised with attenuated influenza and poliomyelitis virus have also revealed small, but significant, increases in SCE as compared to controls. The mechanisms resulting in increased SCE in immunised subjects is unknown, but changes in SCE incidence could reflect changes in the efficiency with which cells take up BrdU or subtle alterations in the proportion of circulating lymphocyte subsets.

Abnormalities in circulating immunoregulatory cells are well established in MS patients. van den Noort and Stjernholm have demonstrated a lymphocytotoxic factor in the sera of MS which inhibits RNA synthesis in stimulated lymphocytes and its titre correlates with disease activity and also with a lowered number of lymphocytes. The relative proportions of T and B lymphocytes have also been shown to vary in MS patients. Oger et al. found an increased proportion of B cells, while others report no change or an increased proportion of T cells. Our own studies on the proportions of T and B cells in these patients, using AET treated sheep RBC to identify rosette forming T lymphocytes, gave high proportions (>80% T cells) in two cases, normal (55 to 65% T cells) in three cases, and low (<55% T cells) in four cases. Recent studies using monoclonal antibodies have shown convincing changes among T lymphocyte subsets in MS patients with a marked reduction in T5/T8 (suppressor-cytotoxic) cells during the active phase of the disease. MS patients have also been shown to be deficient in natural killer cells and NK precursors induced by interferon. There is clear but limited evidence for differences in background SCE frequencies in different subpopulations of lymphocytes so that the evidently altered lymphocyte profile in MS might be considered as the cause for the increased basal levels of SCE in lymphocytes.

The results on SCE incidence in lymphocytes from MS patients and controls exposed in vitro to the mutagens MMC and EMS are summarised in fig 3. The pooled data show a dose dependent increase in SCE frequency for each of the two mutagens, with the SCE incidence in MS cells being consistently

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**FIG 3** Yields of SCE in cultured lymphocytes of nine controls (■—■) and nine MS patients (■—■) following exposure to MMC and EMS in vitro. Each point is the mean value (±SE) from 180 cells.
Spontaneous and mutagen induced sister chromatid exchange in multiple sclerosis

higher than in cells from controls. However, the dose response curves for MS cells are parallel to those of control cells, so that the difference between these two groups is simply a reflection of their basal differences in SCE incidence and is not a consequence of any differential sensitivity to the mutagens. Our findings therefore do not support the suggestion of possible anomalies in DNA repair in MS patients. In conclusion, our evidence would suggest that the enhanced basal SCE frequency in MS lymphocytes is a consistent finding which, on the basis of the mutagen experiments, does not appear to be a consequence of mutagen hypersensitivity associated with abnormal DNA repair. This enhancement would appear to be associated with alterations in lymphocyte population structure, but whether these alterations are themselves a consequence of viral infection remains to be established, and the possibility that the raised SCE incidence is, at least in part, directly rather than indirectly associated with virus infection cannot be discounted.

References


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