Increased translocation frequency of chromosomes 7, 11 and 14 in lymphocytes from patients with neurocysticercosis

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Neurocysticercosis (NCC) has been associated with a high frequency of DNA damage in human circulating lymphocytes and more recently with the development of hematological malignancies. Chronic inflammation, a common feature of helminthic infections, has been proposed to play a key role in carcinogenesis induced by parasites. However, this mechanism is more likely to occur during local tumorigenesis rather than in systemic neoplasia such as that reported for patients with NCC. As an alternative, constant antigen stimulation, which is a feature of chronic NCC, may increase the frequency of aberrations in chromosomes that harbor regions constantly rearranged during T and B lymphocyte maturation, e.g. chromosomes 7 and 14. Therefore, in this study we determined the frequencies of aberrations in chromosomes 7, 11 and 14 in lymphocytes from 10 NCC patients and 10 controls and compared them with the frequency observed in chromosomes 1, 2 and 4 in the same cell samples. Chromosome aberrations were analyzed using a chromosome painting technique. Although the genome painted by probes for chromosomes 1, 2 and 4 was almost twice as large as that painted by probes for chromosomes 7, 11 and 14, translocations involving the latter (median 7.6 per 1000 metaphases) were more frequent than those occurring in chromosomes 1, 2 and 4 (median 2.5 per 1000 metaphases, \( P = 0.002 \)). These results suggest that persistent antigen stimulation can cause chromosome instability in lymphocytes from patients with NCC and should be considered as an additional mechanism whereby parasites may induce cancer.

Introduction

The larval stage, or cysticercus, of the helminth *Taenia solium* causes neurocysticercosis (NCC), the most frequent parasitic disease of the nervous system world wide (Flisser, 1994; Sciutto *et al*., 2000; White, 2000). NCC is a human-to-human infection, acquired by the fecal/oral route from carriers of intestinal *T. solium*, most often in areas with deficient sanitation (Roman *et al*., 2000). Humans are the only definitive host of *T. solium*, 2000). Genetic instability in peripheral lymphocytes can result in an elevated risk for lymphatic and hematopoietic malignancies, as has been indicated by some epidemiological studies with large European cohorts (Bonassi *et al*., 1995; Hagmar *et al*., 1998). Since normal T and B lymphocytes undergo DNA rearrangements to produce functional antigen receptors, these may generate aberrations in some regions of chromosomes that contain the T cell receptor and immunoglobulin loci (Klein, 2000), a situation that could be increased by persistent antigen stimulation, as people are infected with cysticerci. It is endemic in many parts of the world, particularly in Latin America, Africa and Asia (Flisser, 1994; Sciutto *et al*., 2000). It is also frequent in developed countries with high rates of immigration from endemic areas (Richards *et al*., 1985). Currently, NCC is prevalent in the states of California and New Mexico and represents a major cause of morbidity among the immigrant Hispanic population. In Mexico NCC is found in 3% of autopsy material and seroepidemiological studies have reported a prevalence among the general population of 3–12% depending on the zone studied (Sciutto *et al*., 2000). The clinical picture of NCC is heterogeneous and non-specific. Approximately 30–40% of the cases are subclinical. Clinical manifestations vary with the site of infection and host response. Therefore, NCC management may include the anti-parasitic drug praziquantel or albedazole, symptomatic treatment and, in some cases, corticosteroids or even surgical intervention (Flisser, 1994; White, 2000).

A recent epidemiological study reported by our group suggested that NCC is associated with the emergence of malignant hematological diseases; the odds ratio for this association was 3.5, with a 95% confidence interval of 1.2–9.8 (Herrera *et al*., 1999). Several hypotheses have been proposed to explain the association between parasite infections and malignant tumors (Gentile and Gentile, 1994; International Agency for Research on Cancer, 1994; Ohshima and Bartsch, 1994; Rosin *et al*., 1994a). The formation of reactive oxygen and nitrogen species by inflammatory cells, which, aside from killing invading pathogens, induce DNA instability in normal surrounding tissue (Ohshima and Bartsch, 1994; Rosin *et al*., 1994a), a proliferative response by the host cells to repair the tissue damage (Rosin *et al*., 1994b) and altered metabolism of xenobiotics by inflammatory cells, have been proposed as prospective mechanisms by which a chronic inflammatory response contributes to parasite-induced carcinogenesis (Gentile and Gentile, 1994). However, these local effects associated with chronic inflammation do not explain the systemic DNA damage and malignant transformation outside the nervous system in patients with NCC.

We have reported that NCC patients present a high frequency of damage involving chromosomes 1, 2 and 4 in peripheral lymphocytes before treatment, suggesting DNA instability (Herrera *et al*., 2000). Genetic instability in peripheral lymphocytes can result in an elevated risk for lymphatic and hematopoietic malignancies, as has been indicated by some epidemiological studies with large European cohorts (Bonassi *et al*., 1995; Hagmar *et al*., 1998). Since normal T and B lymphocytes undergo DNA rearrangements to produce functional antigen receptors, these may generate aberrations in some regions of chromosomes that contain the T cell receptor and immunoglobulin loci (Klein, 2000), a situation that could be increased by persistent antigen stimulation, as
in the case of NCC. Therefore, in the present study we analyzed the frequencies of aberrations in chromosomes 7, 11 and 14 in lymphocytes from NCC patients and compared them with the frequency observed in chromosomes 1, 2 and 4, which are those most commonly used for genotoxicity studies since they comprise almost 22% of the human genome (Stephens et al., 1990). Although chromosome 11 does not contain any genes encoding antigen receptors, we evaluated aberrations occurring in this chromosome because it is affected in several malignant hematological diseases.

Materials and methods

Individuals

Ten patients with NCC, five women and five men (median age 38 years, range 20–55), and 10 uninfected healthy individuals, five women and five men (median age 35 years, range 20–50) were included in this trial. Blood samples from patients were obtained before therapy. Control individuals were asymptomatic and computer tomography of the brain was used to discard the presence of any silent infection with cysticerci. The Ethical Committee of the Instituto Nacional de Neurología y Neurocirugía approved the protocol. All individuals were informed about the objectives of the present study and written consent was obtained before inclusion in the trial.

Cell cultures

Whole blood (1 ml) was cultured in 9 ml of RPMI culture medium (Sigma, St Louis, MO) supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, NY) and stimulated with 0.4 ml of phytohemagglutinin (Gibco BRL) for 48 h at 37°C. Colcemid (0.3 µg/ml) (Irvine Scientific, USA) was added for the last 3 h of culture. After 20 min 0.075 M KCl hypotonic treatment, cells were fixed with methanol:acetic acid (3:1) and chromosome preparations were made by the standard air drying procedure. The slides were kept in 70% ethanol at –20°C before further use for in situ hybridization (Neubauer et al., 1996).

In situ hybridization

Chromosome aberrations (CA) were analyzed using the three-color chromosome in situ suppression hybridization technique (CISS) for three specific chromosomes (Herrera et al., 2000). CISS is an easy technique which can detect CA as color changes (Lucas et al., 1989; Cremer et al., 1990). Aberrations were analyzed in two codified and separated slides from the same sample of each individual. One slide was hybridized with a cocktail containing DNA probes for chromosomes 1 (red), 2 (green) and 4 (yellow) and the other with DNA probes for chromosomes 7 (green), 11 (red) and 14 (yellow). Aberrations were scored by fluorescence microscopy to determine the frequency of chromosome and chromatid breaks, as well as balanced and unbalanced translocations, in at least 1000 metaphases per individual (Neubauer et al., 1996).

Statistical analysis

The frequency of CA in the infected individuals was compared with the control group using the Mann–Whitney test. The Wilcoxon test for paired samples was utilized to compare results observed in the different sets of chromosomes in both NCC patients and controls. Two-tailed P values of <0.05 were considered significant.

Results

Figure 1 shows the frequency of chromosomal breaks and translocations in peripheral lymphocytes from NCC patients and from a group of uninfected individuals. The determination of aberrations involving chromosomes 7, 11 and 14 indicated a significant difference between patients and controls in the proportion of cells with breaks (P = 0.04) and translocations (P = 0.003). The median frequency of chromosomal breaks was 1 per 1000 metaphases (range 0–2) for controls and 2.5 (range 0–10) for NCC patients, while the median values for chromosomal translocations were 2 (range 0–6) and 7.6 (range 5–10) per 1000 metaphases, respectively. The proportion of cells with breaks involving chromosomes 1, 2 and 4 was higher in NCC patients than in controls (P = 0.02). On the other hand, the comparative analysis of aberrations observed in NCC patients in both sets of chromosomes showed that the frequency of translocations occurring in chromosomes 7, 11 and 14 was significantly different than in chromosomes 1, 2 and 4 (P = 0.002). In both NCC patients and controls translocations affecting chromosome 14 were more frequent (72 and 69% of total chromosome translocations, respectively) than in chromosomes 7 and 11.

Discussion

Taenia solium cysticercosis has been associated with a high frequency of DNA damage in circulating lymphocytes from both infected animals and human patients (Flisser et al., 1990; Herrera et al., 1994, 2000; Montero et al., 1994). In all cases the observed DNA damage returned to normal values after treatment with cestocidal agents. These results suggest an active role of T.solium cysticerci in induction of genetic damage, which could drive the malignant transformation of
host cells. In fact, *T. solium* cystercerosis has been associated with the development of lymphoid tissue malignancies (Herrera et al., 1999) and also of local tumors (Del Brutto et al., 1997). The results of the present study show a higher frequency of chromosomal aberrations in NCC patients compared with uninfected individuals, confirming our previous report (Herrera et al., 2000). The question arises as to how a local infection, such as NCC, induce systemic DNA damage and eventually transform cells outside the central nervous system. Carcino genesis associated with parasite infections has been proposed to result from cell damage linked to a chronic inflammatory process (Ohshima and Bartsch, 1994). However, this explanation is more likely for the development of local tumors rather than for a systemic effect such as that observed in NCC patients. Alternatively, persistent antigen stimulation could give rise to a set of immune cells with genetic instability and chromosome aberrations that ultimately predispose them to malignant transformation. Our results support this possibility, since the frequencies of stable chromosomal aberrations, i.e. translocations, involving chromosomes 7, 11 and 14 were higher than those observed in chromosomes 1, 2 and 4, even though chromosomes 7, 11 and 14 make up only ~13% of the human genome, almost half that of chromosomes 1, 2 and 4 (Stephens et al., 1990). Some of the genes that encode T and B cell receptors are located on chromosomes 7 and 14 and during normal lymphocyte maturation regions of these chromosomes are rearranged or even hypermutated, becoming prone to translocation (Klein, 2000). This process occurs in healthy individuals (Limpens et al., 1995), but it could be increased in chronically infected patients whose immune system continuously implements a response that in most cases does not resolve the infection. Whether the observed differences in the frequencies of aberrations among chromosomes result from a distinct translocation event and/or from expansion of clones carrying a translocation is a question that deserves further investigation. However, chromosome instability resulting from repetitious antigen stimulation should be consid ered as a potential risk factor for the development of hematologi cal malignancies in individuals with chronic NCC.

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References


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