J Headache Pain (2000) 1:S177–S181

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The genetic regulation of SCN in cluster headache may be affected by Th1/Th2 cytokine derangement

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Abstract T lymphocytes participate not only in immune responses but also in the development of several pathologic reactions. Human T helper cells, on the basis of the cytokines they release, fall into two phenotypes, namely TH1 and TH2. The aim of the present research was to assess whether T lymphocytes are involved in cluster headache pathogenesis. We studied 12 cluster headache patients and 6 control cases in terms of T lymphocyte subsets and their related cytokine release. Our results show that during a cluster headache attack, the TH1 subset is activated, whereas in the periods between and out of the crisis, the TH2 cells become predominant. The possible role played by T lymphocytes and cytokines in the biomolecular phenomenon leading to cluster headache is discussed.

Key words T lymphocytes • Cytokines • Cluster headache • Genetic clock regulation

Introduction

The existence of polarised human T cell responses, reminiscent of the TH1 and TH2 subsets already described for mouse T cells, is well documented [1]. TH1 and TH2 responses not only play different roles in protection, but they can also promote different immunopathological reactions.

TH1 cells produce interferon (IFN)-γ, interleukin (IL)-2 and tumour necrosis factor beta. On the other hand, TH2 cells release interleukin-4, IL-5, IL-6, IL-9, IL-10 and IL-13 [2]. TH1 cells are responsible for cell-mediated immune responses, for macrophage activation in terms of antibody-dependent cell cytotoxicity (ADCC) and for delayed-type hypersensitivity (DTH). TH2 cells provide optimal help for humoral immune responses. These include IgE and IgG isotype switching, mucosal immunity through activation of mast cells and eosinophils, and facilitation to IgA synthesis [3]. Human TH1 and TH2 cells differ not only in their different lymphokine secretion profiles, but also in their responsivenesses to lymphokines. Both TH1 and TH2 cells proliferate in response to interleukin-2, but TH2 are much more responsive to IL-4 than are TH1 cells [4]. IFN-γ plays a selective inhibitory effect on the proliferative response of TH2 cells [5]. Moreover, human TH1 cells preferentially develop during infections by intracellular bacteria, protozoa and viruses [6, 7], whereas TH2 cells predominate during helminthic infections [8] and in response to common environmental allergens.

TH1 and TH2 type cytokines are mutually inhibitory for the differentiation and the effector functions of the reciprocal phenotypes. Thus, a strong TH2-oriented response tends to downregulate TH1-type responses and vice versa [9]. A wide variety of infections and autoimmune diseases is characterised by responses that are strongly polarised to either TH1 or TH2 patterns of cytokine secretion.

Cluster headache is characterised by a precise timing of crises during both the span of the day and during the year, one-sided pain, and great autonomic disturbances. Little is known about its aetiology and pathogenesis. Most cases of cluster headache are sporadic, but an autosomal dominant gene is involved in 3%–4% of women and 7%–10% of men with cluster headache [10, 11]. Changes in immunologically competent cells, such as natural killer lymphocytes, monocytes, and helper and suppressor T lymphocytes have been reported in cluster headache, both during the active period and in remission [12].

The hypothalamic area claimed to be the physiopathological core of cluster headache is anatomically close to the suprachiasmatic nucleus (SCN) that regulates the phenotype of mammalian clocks via modulation of TH1 and TH2 subsets and their subsequent cytokine production [13, 14].

We analysed cluster headache as an immunologic disorder by studying the TH1 and TH2 subsets and their cytokine secretion profiles.

Material and methods

We studied 12 cluster headache (CH) patients who met the International Headache Society criteria for the diagnosis of episodic cluster headache [15]. The mean age of the patients was 34 years (range, 21 to 45 years); the mean disease duration was 12.6 years. None of the patients had received any prophylactic or rescue drug prior to the sampling. Control cases consisted of a total of 6 age- and sex-matched healthy individuals.

The local Ethics Board approved the study protocol and informed consent was obtained from all patients and controls prior to the study.

Blood collection was carried out at three different times: during a spontaneous cluster headache episode, between CH episodes and outside the florid cluster phase.

We isolated peripheral lymphocytes to determine by flow cytofluorimetric analysis the TH1 and TH2 subsets, following the method previously described [16]. At the same time, a quantitative cytokine assay was carried out on serum of both patients and controls with commercial immunoenzymatic assays.

Results

During cluster headache attacks, the serum levels of IFN-γ and IL-2 significantly increased not only with respect to controls but also with respect to the other times analysed in the same CH patients (Figs. 1, 2). In fact, between and out of the crises, the IFN-γ and IL-2 values in CH patients were similar to controls. At the same time, the cytofluorimetric analysis, through an intracellular staining for IFN-γ, showed a clear-cut TH1 profile (Fig. 3). Therefore, during a cluster headache crisis, TH1 cells are activated and predominant over the TH2 subset.

The serum levels of TH2-type cytokines, namely IL-4, were dramatically increased in CH patients in the periods between and out of the crises (Fig. 4). There was not any significant difference between control values and CH patients during the crises. Even in this case, the cytofluorimetric analysis paralleled with the serum data demonstrating, through an intracellular staining for IL-4, an increase in the TH2 subset (Fig. 5). Therefore in the periods between and out of cluster headache crises, the TH2 cells become activated and predominant over the TH1 subset.

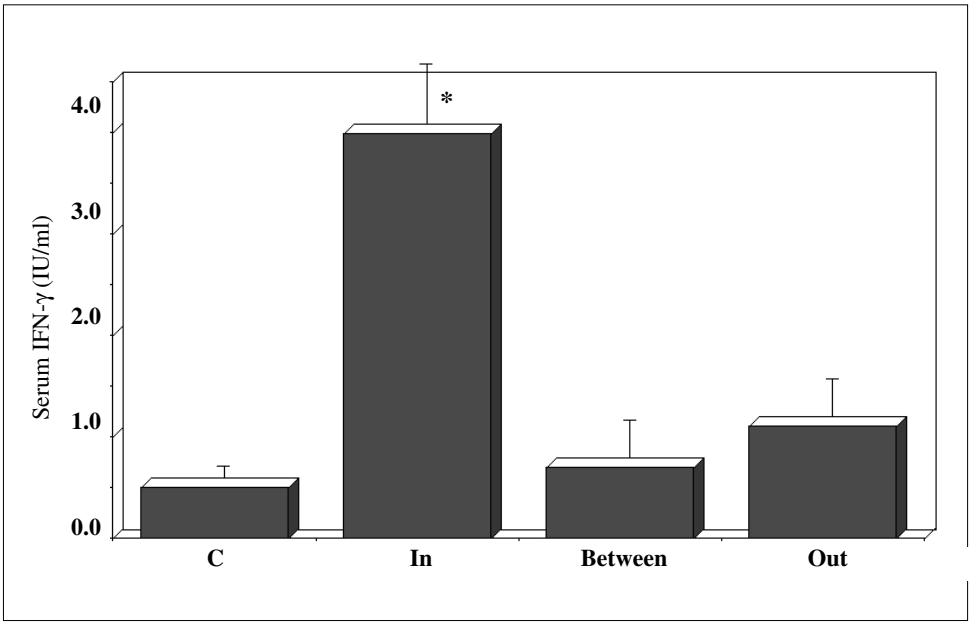

Fig. 1 Mean serum levels (± SEM) of IFN-γ as assayed by EIASA in 12 CH patients studied during the attack (*In*), in between attacks (*Between*), out of florid phase (*Out*) and in 6 controls (*C*). *$p < 0.003$ In vs. C, Between and Out

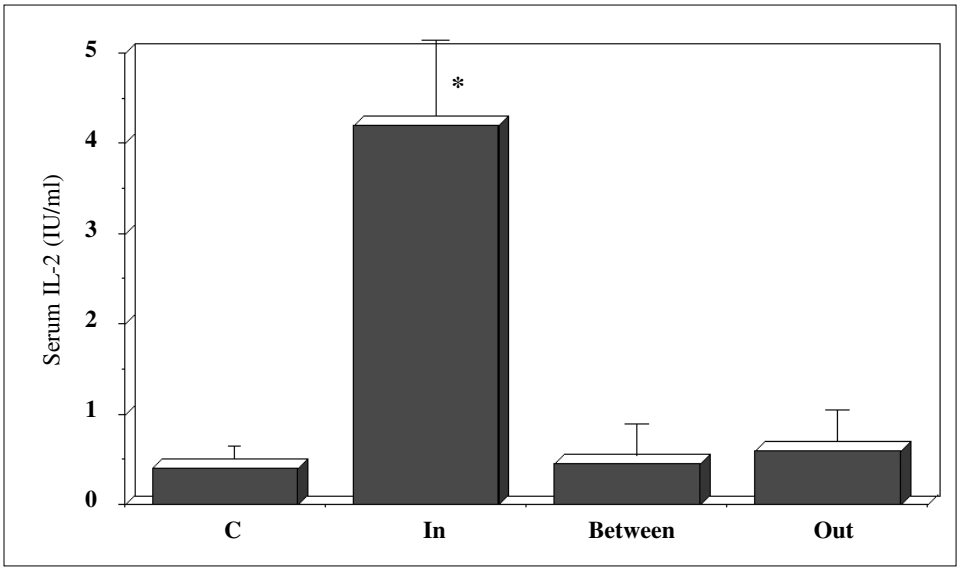

Fig. 2 Mean serum levels (± SEM) of IL-2 as assayed by EIASA in 12 CH patients studied during the attack (*In*), in between attacks (*Between*), out of florid phase (*Out*) and in 6 controls (*C*). *$p < 0.01$ In vs. C

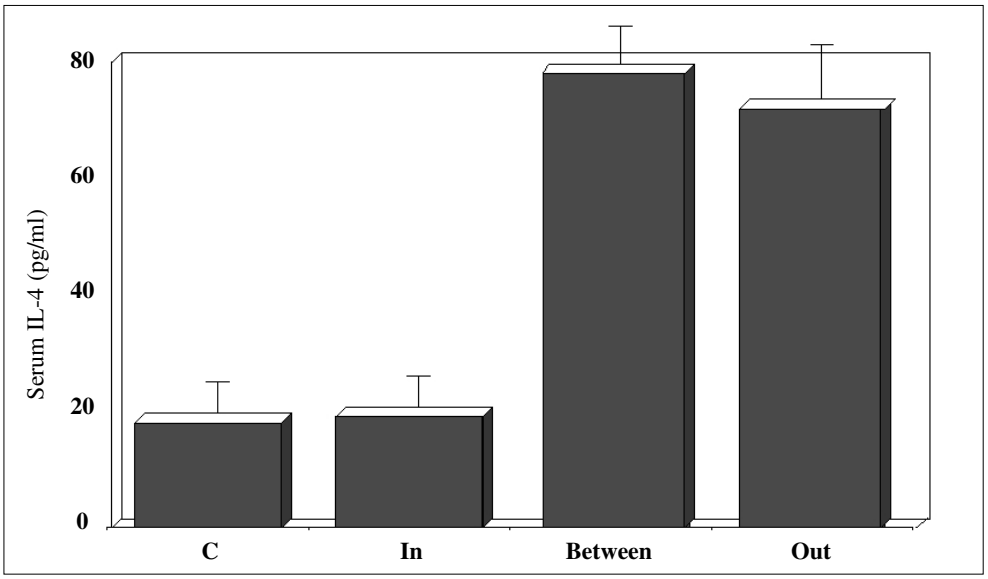

Fig. 3 Mean serum levels (± SEM) of IL-4 as assayed by EIASA in 12 CH patients studied during the attack (*In*), in between attacks (*Between*), out of florid phase (*Out*) and in 6 controls (*C*). * $p < 0.02$ C vs. In

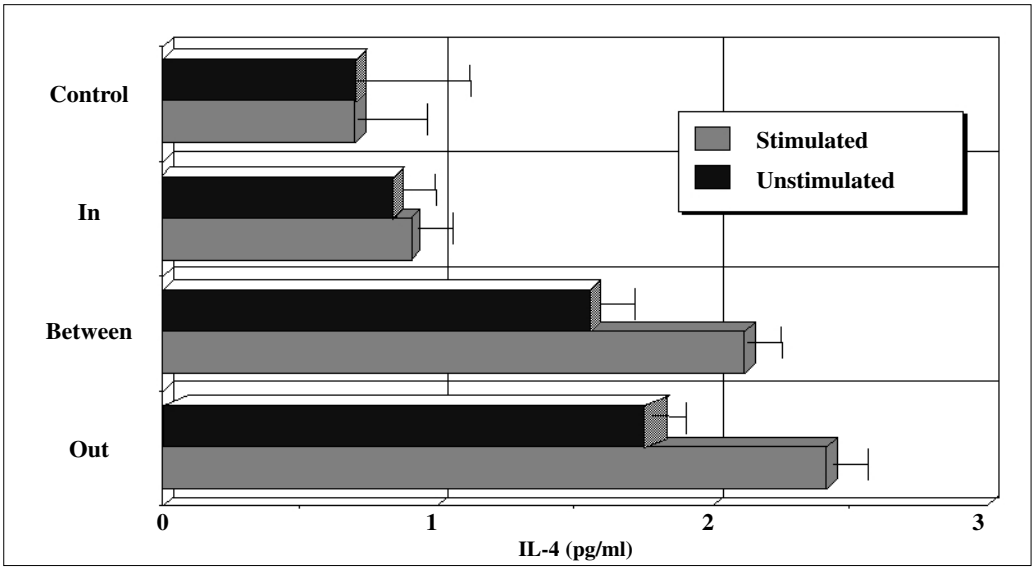

Fig. 4 Mean values (± SEM) of TH2 IL-4 synthesis as assayed by cytofluorimetric tri-colour fluorescence determination is significantly increased in both CH Out and Between phases if compared to controls ($p < 0.001$). *Stimulated*, with phorbolmyristate acetate (PMA) and ionomycin

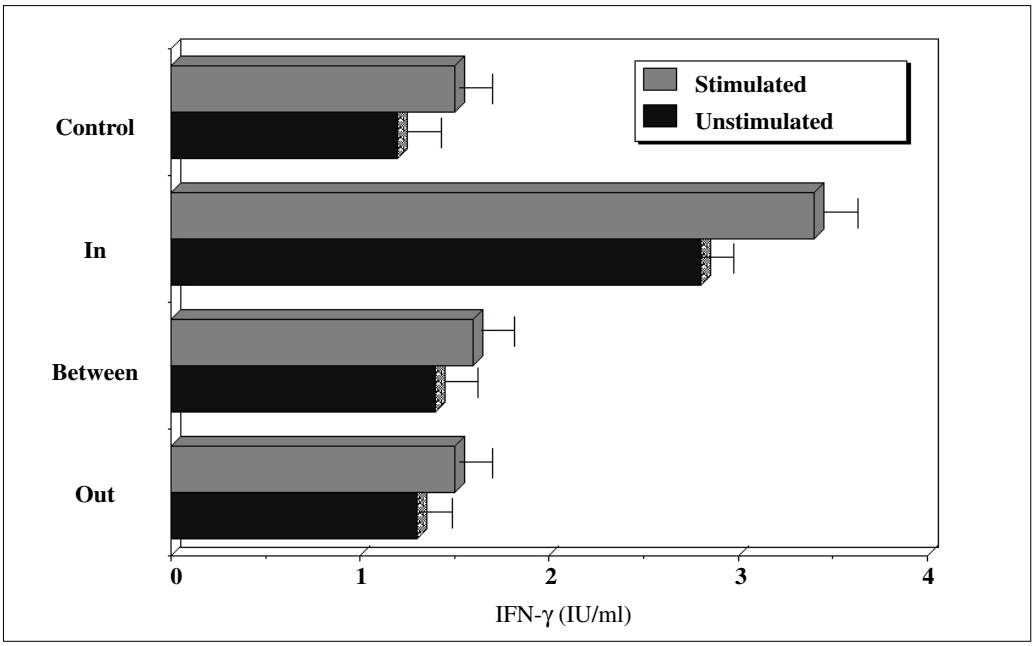

Fig. 5 Mean values (± SEM) of Th1 IFN-γ synthesis as assayed by cytofluorimetric tricolour fluorescence determination is significantly increased in both CH In and Between phases if compared to controls ($p < 0.004$). *Stimulated*, with PmA and ionomycin

We did not find any significant correlation between the mean value of the studied cytokines sampled at the different times of the day.

Discussion

Our data show that TH1 and TH2 type cytokines, as well as TH1 and TH2 lymphocyte subsets, are involved in the biomolecular phenomenon leading to cluster headache. Since the regulation of the suprachiasmatic nucleus oscillator is controlled by these cytokines, any modification of TH1 and TH2 subsets could be responsible for suprachiasmatic nucleus desynchronization. This may illuminate our understanding of the basic mechanism of cluster headache attack rhythms [17]. Finally, our data are useful for the introduction of new immunotherapeutic protocols in cluster headache treatment.

Acknowledgements This work has been supported by grants from MURST 1995 and by the Italian League of Headache Sufferers. The authors thank Professor Charalambos Panayotis Kyriacou for his valuable suggestions and Dr. Mario Falciano for support in the realisation of the study results.

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