



Review

Immunological findings in patients with migraine and other primary headaches: a narrative review

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Summary

Experimental findings suggest an involvement of neuroinflammatory mechanisms in the pathophysiology of migraine. Specifically, preclinical models of migraine have emphasized the role of neuroinflammation following the activation of the trigeminal pathway at several peripheral and central sites including dural vessels, the trigeminal ganglion, and the trigeminal nucleus caudalis. The evidence of an induction of inflammatory events in migraine pathophysiological mechanisms has prompted researchers to investigate the human leukocyte antigen (HLA) phenotypes as well as cytokine genetic polymorphisms in order to verify their potential relationship with migraine risk and severity. Furthermore, the role of neuroinflammation in migraine seems to be supported by evidence of an increase in pro-inflammatory cytokines, both ictally and interictally, together with the prevalence of Th1 lymphocytes and a reduction in regulatory lymphocyte subsets in peripheral blood of migraineurs. Cytokine profiles of cluster headache (CH) patients and those of tension-type headache patients further suggest an immunological dysregulation in the pathophysiology of these primary headaches, although evidence is weaker than for migraine. The present review summarizes available findings to date from genetic and biomarker studies that have explored the role of inflammation in primary headaches.

Keywords: headache, neuroinflammation, immunology, genetics, biomarkers

Abbreviations: CRP, C-reactive protein; CAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; CH, cluster headache; CCR2, Chemokine (C-C motif) Ligand 2 (CCL2) receptor; CREB, CAMP response element binding protein; CSD, cortical spreading depression; CTLA-4, cytotoxic T lymphocyte antigen 4; DCs, dendritic cells; ERK, extracellular-signal-regulated kinase; FOXP3, forkhead box protein 3; GWAS, genome-wide association; HLA, Human leukocyte antigen; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, Interleukin; LTA, Lymphotoxin A; MA, migraine with aura; MAPK, mitogen-activated protein kinase; MCs, mast cells; MEFV, Mediterranean fever gene; MHC, major histocompatibility complexes; NO, nitric oxide; onabotA, onabotulinumtoxin A; PCR, polymerase chain reaction; Treg, regulatory T; SNPs, single nucleotide polymorphisms; TG, trigeminal ganglion; TGF, transforming growth factor β 1; TLR, toll-like receptors; TNC, trigeminal nucleus caudalis; TNF, tumor necrosis factor α ; TTH, tension-type headache; VCAM-1, vascular cell adhesion molecule-1

Introduction

Over the last decades, the role of neuroinflammation in primary headache pathophysiology has been increasingly recognized [1]. Indeed, data from pre-clinical models clearly support an involvement of pro-inflammatory cytokines and immune cells, both in the induction and in the maintenance of headache. Most of these findings have been related to migraine, with significantly less for cluster and tension-type headaches. In the Fig. 1a–c, the main putative neuroinflammatory pathogenetic mechanisms of primary headaches are represented.

Migraine is a primary headache characterized by attacks with pulsating features of moderate to severe intensity usually affecting one side of the head and typically lasting from 4 to 72 hours. It is frequently accompanied by nausea, vomiting,

phono, and photophobia and generally it tends to worsen due to physical activity [2].

In the field of migraine pathophysiology, current neurovascular theory points to the hypothalamus as attack origin site [2]. Hypothalamic activation is responsible for the stimulation of trigeminal nucleus caudalis (TNC) that, in turn, stimulates trigeminal ganglion (TG). In light of this activation, calcitonin gene-related peptide (CGRP) is released from trigeminal c fibers surrounding pial and dural vessels and, via interaction with own receptors, leads meningeal vessel dilation and nociception [3].

In pre-clinical models of migraine, the activation of trigemino-vascular system induced by electrical stimulation of TG has been shown to cause a local neurogenic inflammation at the

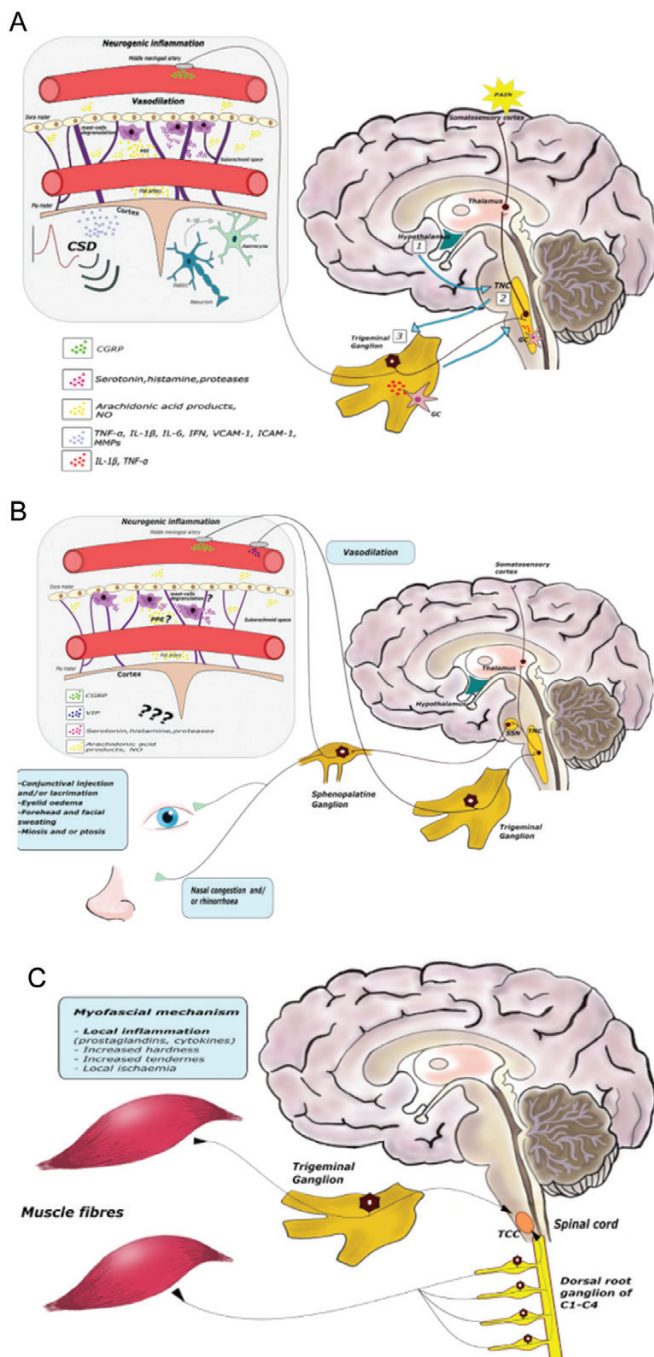


Fig. 1. (a) Neuro-inflammatory mechanisms involved in migraine. According to current neurovascular theory, primum movens of migraine attacks is hypothalamic activation (1), which is responsible for the stimulation of trigeminal nucleus caudalis (TNC) (2) that, in turn, stimulates trigeminal ganglion (TG) (3). CGRP released from trigeminal endings of TG as a consequence of their activation induces, at least in rats, plasma protein extravasation (PPE) together with morphological changes and activation of dural mast cells. Activated mast cells release a variety of inflammatory mediators such as histamine, serotonin, proteases, nitric oxide (NO) and some arachidonic acid products and pro-inflammatory cytokines including IL-1, TNF- α , and IL-6. These inflammatory mediators contribute to the activation of dural trigeminal fibers. Preclinical data suggest that CGRP released from the soma or local axon varicosities is able to induce the release of some pro-inflammatory cytokines and NO from trigeminal ganglion glial cells. These substances can enhance CGRP release, creating a positive feedback loop within the ganglion, therefore contributing to trigeminal pain transmission. From TG pain impulses come back to TNC,

dural vessels. This involves a plasma protein extravasation as the result of an increased meningeal vascular permeability [4]. In addition to dural extravasation, electrical stimulation of the TG produces morphological changes associated with inflammation, involving activation of mast cells (MCs) in close proximity to the dural autonomic nerves and sensory afferents that express CGRP [5] as well as platelet aggregation within the dural vasculature [6]. Activated MCs, in turn, release several mediators including serotonin, histamine, heparin, proteases, and arachidonic acid products and generate pro-inflammatory cytokines and chemokines. All of these mediators therein contribute to the sensitization of trigeminal endings [7]. Besides MCs, other cells are believed to contribute to meningeal neurovascular inflammation for migraine. For instance, activated macrophages are able to release pro-inflammatory cytokines. Cytokines and chemokines locally produced from these resident immune cells and other inflammatory molecules may function as relevant pain mediators [7, 8]. Also, dendritic cells (DCs) along with T lymphocytes (for the most part memory CD4+ and CD3+ T cells) in the dural vessels, and in the subarachnoid space, have been reported as putative player in the pathophysiology of migraine [9, 10]. This is supported by evidence from experimental models of their role in mediating neuropathic and inflammatory pain [11, 12].

Besides dural vessels, in the trigeminal pathway also other sites are characterized by neuro-inflammatory events. In the TG, CGRP binds to A δ trigeminal ganglion neurons

and from TNC, they are transmitted to thalamus, which in turn projects to somato-sensory cortex, where conscious nociception occurs. An increase in the expression of several pro-inflammatory cytokines has also been reported as a direct consequence of cortical spreading depression (CSD), which is considered the pathophysiological substrate of migraine aura. CSD may trigger neuroinflammation via the pannexin-1 (Panx1) channel opening and subsequent caspase-1 activation, which is responsible for the cleavage of pro IL-1 β and pro-IL-18. These cytokines, in turn, activate parenchymal neuro-inflammatory signalling and nuclear factor κ B activation in astrocytes. (b) Putative neuro-inflammatory mechanisms involved in cluster headache. The pathophysiological mechanisms of cluster headache include activation of the trigemino-vascular and the parasympathetic nervous systems, which are responsible for the excruciating pain and autonomic signs/symptoms, respectively. Posterior hypothalamus via the interaction with several components of the trigemino-vascular system is believed to play a pivotal role as a potential generator, or better a potential pain modulator in this primary headache disorder. No experimental animal models of cluster headache aimed to investigate the involvement of neuro-inflammatory mechanisms are available so far. However it can be hypothesized that neurogenic inflammation might also occur as a consequence of dural trigeminal and parasympathetic fiber activation and the subsequent CGRP and VIP release. Data are also lacking on the cross talk between glial cells and trigeminal fibres in the trigeminal ganglion in the context of cluster headache, as well as no data are available on the cross talk between glial cells and parasympathetic fibres in sphenopalatine ganglion and superior salivary nucleus (SSN). (c) Putative neuro-inflammatory mechanisms involved in tension-type headache. Until now, the precise role of neuroinflammation in tension-type headache pathophysiology is not well understood. However, a local muscle inflammation is believed to contribute to activate myelinated (A δ) and unmyelinated (C) fibres. From myofascial nociceptors, pain impulses are transmitted both to trigeminal ganglion and dorsal root ganglion of C1-C4. These structures, in turn, activate trigemino cervical complex (TCC). Finally, TCC project to somato-sensory cortex via thalamus within pain network. CSD, cortical spreading depression; CGRP, calcitonine-gene-related peptide; ICAM, intracellular adhesion molecule; IL, interleukin 1; MMP, matrix metalloproteinase; NO, nitric oxide; Panx1, pannexin-1; PPE, plasma protein extravasation; TNC, trigeminal nucleus caudalis; TG, trigeminal ganglion; TCC, trigeminal cervical complex; TNF, tumor necrosis factor-alpha; VIP, vasoactive intestinal peptide; VCAM, vascular adhesion molecule.

expressing CGRP receptors facilitating nociceptive transmission to second-order neurons in the TNC. The interaction of CGRP with its receptor on these fibres contributes to amplifying nociceptive signalling. Activated trigeminal fibres transmit nociceptive messages to the TG, and antidromic axon reflexes are believed to induce a further release of CGRP within the dura, therein increasing blood flow to the region [13]. Sensitization of the trigeminal pathway, both peripherally and centrally, has been associated to the stimulation of intracellular signalling molecules relevant to pain, including cyclic adenosine monophosphate (cAMP), its response element binding protein (CREB), the mitogen-activated protein kinase (MAPK) p38, and extracellular-signal-regulated kinase (ERK) [14].

Resident glial cells also possess CGRP receptors. The interaction of CGRP with its receptors on these cells stimulates the production of some pro-inflammatory cytokines including tumor necrosis factor (TNF)- α and interleukin (IL)-1, both of which amplify trigeminal nociception [13]. Moreover, neuro-inflammatory events involving microglia and astrocytes also occur in the course of cortical spreading depression (CSD), which is considered the pathophysiological substrate of migraine aura [6]. Several studies have shown that CSD increases the expression of pro-inflammatory cytokines such as TNF- α , IL6, IL-1 β , interferon (IFN)- γ , as well as vascular cell adhesion molecule-1 (VCAM-1), chemokine (C-C motif) ligand, and intercellular adhesion molecule-1 (ICAM-1) [15–18]. Increased levels of toll-like receptors (TLR3 and TLR4) due to CSD have also been reported [19].

Based on the putative role of neuroinflammation in migraine coming from experimental animal models, several researchers investigated for changes in cytokine levels and lymphocyte subsets in migraineurs, both in headache-free periods and during attacks.

Additional studies have focused on specific polymorphisms of genes encoding some pro-inflammatory cytokines and loci within human leukocyte antigen (HLA) regions in order to test for any association with migraine risk and severity [20].

Neuro-inflammatory mechanisms have also been advocated for cluster headache (CH), a primary headache characterized by recurrent very severe pain attacks involving one side of the head, generally around the eye. Attacks typically last from 15 to 90 minutes and very often are accompanied by autonomic features, such as nasal congestion, eye watering, or swelling around the eye [2, 21]. Although the precise aetiology of this disorder is unknown, it is widely accepted that a prominent pathophysiological role is played by the activation of the trigemino-vascular system. Specifically, a trigemino-parasympathetic reflex is considered to play a major role in the pathogenic mechanisms of CH. This reflex is responsible for the unilateral severe pain in the first trigeminal branch territory and associated autonomic symptoms [22]. The activation of the trigemino-vascular system could in turn induce the secretion of several neuro-inflammatory mediators, including CGRP, nitric oxide (NO), and neurokinin A, thus amplifying nociception [23, 24].

In support of a role of neuroinflammation in this disorder, changes in cytokine levels and in some immune cell subsets have been detected in CH patients.

Tension-type headache is the most frequent primary headache and is characterized by mild-to-moderate pain having variable duration, generally bilateral and of pressing or tightening quality [2]. The pathogenesis of tension-type headache

(TTH) is multifactorial, involving both peripheral (myofascial nociception) and central (failure of endogenous pain control) mechanisms [25]. Limited data are available regarding any involvement of the immune system and neuroinflammation in this primary headache type.

In the present review, we will focus on genetic and peripheral markers referring to the immune system achieved from human studies which have tested the neuro-inflammatory hypothesis of primary headaches pathogenesis. Specifically, we will summarize available findings exploring a role of inflammation and immunological dysfunction in migraine, CH, and TTH patients, since, to the best of our knowledge, an up-date overview on evidence in this regard is lacking.

Genetic findings related to the immune system in migraineurs

Over all, data deriving from peripheral blood samples of primary headache patients concern general genetic changes, not linked to specific migraine mechanisms but instead to inflammatory and immune system responses. Genetic research on migraine and, to a lesser extent CH, have focused mainly on HLA distribution and polymorphisms of genes encoding for relevant molecules in the immune system, notably cytokines.

Some genetic susceptibility factors for migraine within the HLA region have been investigated. The HLA complex is characterized by a high polymorphism and plays a pivotal role in human adaptive immune responses. HLA encodes class I and class II major histocompatibility complexes (MHCs), which are specifically involved in antigen presentation to CD8+ and CD4+ T cells, respectively [26].

One of the first studies to investigate for a possible link between migraine and HLA polymorphisms reported that HLA Class I A, B, C antigens have similar frequency distributions in both migraineurs and controls. Whereas, a reduced frequency of the HLA Class II DR2 antigen has been observed in migraine with aura (MA) compared to migraine without aura (MwA) and controls, suggesting a potential protective role from DR2 antigen against MA onset [27].

Another research focusing on the distribution of HLA-DRB1 alleles in a cohort of Italian migraine patients, reported a reduced frequency for DRB1*12 allele, while an increased frequency for DRB1*16 allele in patients with migraine compared to controls was found. Subgroup analyses evidenced a significant increase for the HLA-DRB1**16 allele only in MwA [28].

Most past studies investigating HLA-migraine correlations have had limitation regarding their methodology. Specifically, HLA has been typed by means of low resolution technique, including antibody-based microlymphocytotoxicity tests and polymerase chain reaction (PCR) using specific probes and primers [27, 28]. Furthermore, studies have focused on a limited number of HLA loci. Finally, because of their small sample sizes, no reliable associations were detected due to insufficient statistical power [29].

A case-control study carried out by Huang *et al.* in 2020, including a large cohort of 2999 cases and 6055 controls, sought to overcome these limitations, by using a high-resolution technique [30]. In this study, a significant association between HLA-B and C and clinic-based migraine was reported. Specifically, age and sex-adjusted odds ratios for HLA-B*39:01, HLA-B*51:01, HLA-B*58:01, and HLA-C*03:02 were 1.80, 1.50, 1.36, and 1.36, respectively.

Furthermore, HLA-B*58:01 or HLA-C*03:02 migraine carriers had 1.63-fold probability of suffering chronic migraine.

As far as cytokine genetic polymorphisms are concerned, particular attention was devoted to TNF family because of its contribution to neuroinflammation induced by trigemino-vascular activation and its activating effect on CGRP transcription. The TNF- α -308G/A polymorphism was mostly investigated in migraine. This polymorphism is related to some autoimmune, infectious, and neoplastic diseases and has been shown to increase *in vitro* TNF- α production [31, 32]. An association between this polymorphism and migraine continues to be debated due to inconsistent results and small samples sizes of the studies investigating this association [33–40].

A systemic review and meta-analysis conducted by Schürks *et al.* and published in 2011, including 10 studies, failed to report an overall association between the TNF α and TNF β gene variants, and migraine. However, subgroup analyses revealed that the A allele of the TNF α -308G > A variant was associated to a more than doubled risk for migraine, mainly for MwA (pooled OR = 2.87) in populations with different ethnicities, with a greater effect among females. In addition, the risk for MA was greater among Asian populations (pooled OR = 1.71) and this effect resulted to be stronger in females [41].

A further meta-analysis of five studies on Asian populations, including 985 cases and 958 controls, reported an association between TNF- α -308G/A polymorphisms and migraine risk (with ORs of 1.73 for adenine (A) vs. guanine (G), 1.78 for GA vs. GG and 1.82 for AA+GA vs. GG, respectively). Subgroup analyses revealed statistically significant results for MA (OR: 1.72 for GA vs. GG and 1.65 for AA+GA vs. GG) but not for MwA [42].

An additional meta-analysis carried out by Chen *et al.* in 2015 included 11 studies for an overall number of 6682 Caucasian and non-Caucasian migraineurs and 22 591 controls and investigated the correlation between TNF -308 G>A and migraine. Despite denying an overall association between this polymorphism and migraine risk, subgroup analyses demonstrated, in line with previous results, that the 'A' allele of the TNF -308G>A variant increases the risk of migraine among non-Caucasians (pooled OR = 1.82). Moreover, the risk for MA was slightly increased among both Caucasians and non-Caucasians [43].

Likewise, Fawzi *et al.* (2015) found that increased migraine risk was associated with TNF- α -308 GA, AA genotypes and A allele, as well as both TNF- α -857 CT genotype and T allele. In this study, TNF- α levels resulted increased in patients with migraine compared to controls [44].

Lymphotoxin A (LTA), known also as TNF- β , is another member of the TNF cytokine superfamily which is considered a relevant factor in migraine susceptibility. TNF- β gene is mapped on chromosome 6; it plays a key role in immune and inflammatory responses and some polymorphisms of this gene have been reported to have regulatory function in TNF- β levels. In particular, the TNF- β 252A.G polymorphism (rs909253) is a variant with a silent point mutation with the capacity to modifying gene expression [45, 46].

Studies investigating the association between TNF- β 252A.G polymorphism and migraine risk have led to conflicting results. This association was firstly reported by a study involving an Italian population, where the carriage of the A allele conferred a high risk for MwA [47]. Other studies in this regard showed contrasting results [37, 48, 49].

A meta-analysis of seven studies, including 5557 migraineurs and 20 543 unrelated healthy controls, did not find any significant association between TNF- β 252A.G polymorphism and overall migraine risk. Stratified analyses according to by migraine subtypes and sex revealed similar results. Once again, an increased migraine risk was found for Asians under the recessive model (GG vs. AG + AA: OR, 1.38; 95%CI, 1.04–1.84; *P* for heterogeneity, 0.665) [50].

Only a single study has explored the association between specific TNF polymorphisms and specific phenotypic characteristics of migraine. This study reported that TNF- β G252A gene, as well as monoamine oxidase A T941G polymorphisms, are associated to photophobia but not to osmophobia in migraine patients [51].

Additional genetic LTA variants rs2009658, rs2844482, and rs2229094 were identified in a p genome-wide-association study (GWAS) conducted in the Norfolk Island population as associated with migraine: *P* = 0.0093, *P* = 0.0088, and *P* = 0.033, respectively. The same single-nucleotide polymorphisms (SNPs) were genotyped in a large case-control Australian Caucasian population and tested for an association with migraine but none were associated with migraine in this cohort [52]. Furthermore, in a study on Jordanian population, rs1800629, rs1799724, and rs1799724 polymorphisms in TNF α , but not rs909253 polymorphism in LTA, were significantly associated with migraine [53].

Further investigations on the role of TNF SNPs as potential predictors of migraine risk provided contrasting results. Specifically, a large case-control cohort investigating the role of the TNF gene cluster in migraine did not report any significant associations of 9 SNPs with migraine subtype and gender [27].

Conversely, in another research where a total of 37 variants distributed across 14 genes were genotyped, it was reported that SNPs rs9371601 and rs3093664 in the spectrin repeat containing nuclear envelope protein 1 (SYNE1) and TNF genes, respectively were associated with menstrual migraine [54].

A few studies investigated for the contribution of other candidate genes related to other cytokines to migraine pathophysiology. To this regard, an investigation by Rainero *et al.* in 2002 focused on the putative association of the -889 C/T biallelic polymorphism of the IL1 α gene with different clinical characteristics of the disease. In this study, migraine patients carrying the T/T genotype had a significantly lower age at disease onset compared to IL1 α C/C or C/T carriers (*P* < 0.01). Additionally, the same genotype was significantly more frequent in patients with MA than in those patients with MwA (*P* < 0.05) [55]. Noteworthy, TT polymorphism had been already found to be associated with age of onset for Alzheimer's disease and rheumatoid arthritis [56–58].

Furthermore, Yilmaz *et al.* revealed significant differences in the genotypic distributions between migraineurs and controls not only for TNF- α -308 G/A but also for IL-1 β +3953 C/T polymorphism (*P* = 0.004). Specifically, the authors found a significant increase in the prevalence of IL-1 β +3953 T allele in MwA (*P* = 0.004) [38].

Regarding IL-6, a study by Rainero *et al.* enrolling 268 migraine patients and 305 control subjects investigated for an association between migraine and the -174 G/C IL-6 polymorphism. The latter had already been reported to be correlated with higher IL-6 plasma level. However, no significant

correlation between the above polymorphism and the migraine risk was found [59].

An additional study focused on A/G polymorphism located within exon 1 of the gene encoding the cytotoxic T lymphocyte antigen 4 (CTLA-4) which is involved in several HLA-associated multifactorial diseases and exerts a negative control on T-cell proliferation and cytokine production such as TNF- α and IL-10 [60]. Results showed no statistical difference in allele frequencies between patient groups (migraine with and without aura) and controls.

Another investigation focused on eight common missense mutations of Mediterranean fever gene (MEFV) encoding the pyrin protein. This pyrin is involved in the regulation of inflammatory activity and in the processing of pro-IL-1 β . Results indicated that biallelic mutations in MEFV gene were correlated with an increased risk of migraine in the Turkish population. Furthermore, MEFV mutations appeared to be related to a higher frequency and shorter durations of migraine attacks [61].

Concerning the genetic basis of migraine comorbidities, an association study investigated 77 polymorphisms potentially related to cardiovascular disorders and migraine. It reported that variants in TNF, the Chemokine (C-C motif) Ligand 2 (CCL2) receptor (CCR2), transforming growth factor (TGF) β 1, nitric oxide synthase (NOS)3, and IL9 were associated with migraine but these associations did not remain significant after adjustment for multiple testing [62].

Finally, in a meta-analysis of 375 000 individuals, 38 susceptibility loci were identified for migraine. Among these, some loci were linked to relevant immunological mechanisms, especially the control of inflammation, namely TGF β receptor 2 (R2) rs 6791480, and nitric oxide signalling, i.e. REST, GJAI, YAP, PRDM16, LRP1, and MRV11 [63].

As far as TGF β is concerned, evidence supports the involvement of this pleiotropic cytokine not only in immune regulation by exerting a powerful anti-inflammatory function, but also in the regulation of the homeostasis for several tissues [64, 65].

Its role is not limited to immune cell function regulation but also to the regulation of vascular tone and reactivity by inhibiting the production of potent vasodilators such as NO and by stimulating the production of potent vasoconstrictors such as endothelin [66, 67]. Variations in the levels of these substances or end products have been evidenced in migraineurs especially during attacks, but their possible association with TGF- β 1 levels or genetic polymorphisms have never been investigated.

In conclusion, genetic findings related to immune dysfunction in migraine are conflicting. Possible factors of inhomogeneity may be associated with different study techniques used for genes typing and different sample sizes.

Despite these limitations, genetic findings suggest that some specific polymorphisms related to HLA may be associated to migraine, particularly HLA-B and C.

Among studies on cytokine polymorphisms and SNPs, only an association between TNF- α -308G/A polymorphism and migraine was confirmed but limited to Asian population, whereas an association between TNF- β 252A G polymorphism and overall migraine risk was denied by the majority of studies investigating this association. Other positive findings are the association of migraine with IL-1 IL-1 β +3953 C/T as well as the association of a lower migraine

age of onset and IL-1 α -889 T/T genotype but these findings have been not replicated. Finally, a single study reported that TGF- β receptor 2 rs 6791480 may be a susceptibility gene factor for migraine.

Figure 2 summarizes the main findings on cytokine polymorphisms associated to an increased migraine risk or an early age of migraine presentation.

Cytokine levels in migraineurs

Several studies have investigated cytokine profile in migraineurs. Significant increases in the peripheral levels of pro-inflammatory cytokines, whose role in many autoimmune disorders is well known, have been shown to be present in migraine patients, both in interictal and ictal periods. In particular, increases in TNF- α , IL-1 β , IL-6, and IL-12p70 have been reported interictally [68–71], as well as high peripheral levels for the pro-inflammatory chemokine IL-8 [70]. Conversely, levels of the anti-inflammatory cytokine IL-10 have been found to be similar [72] or even decreased between attacks [69, 71, 73, 74] in patients with migraine, when compared with controls. Among cytokines exerting an anti-inflammatory effect only for TGF- β 1 plasma levels were found to be increased in migraineurs interictally [75].

Chemokines IL-8 and chemokine (C-C motif) ligand 3, the latter also known as macrophage inflammatory protein 1 (CCL3/MIP-1a), have been reported to be higher in migraineurs when assessed interictally [70]. During attacks, significant further increases in the peripheral levels of pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 have been observed, whereas levels of IL-4 and IL-5 as well as soluble ICAM 1 have been decreased [76, 77]. A research reported that serum levels of IL-1 β , IL-6, TNF- α , and CGRP in patients with migraine were significantly higher than controls, and CGRP levels in migraineurs assessed interictally were significantly correlated with IL-1 β and IL-6, but not with IL-2, IL-10, and TNF- α levels. These results suggested that IL-1 β and IL-6 may have been related to the pathogenesis of migraine attacks and CGRP might be related with the secretion of the above cytokines [78].

The relevance of pro-inflammatory cytokines and chemokines in migraine, especially between attacks, suggests an underlying pro-inflammatory status in migraine, and a Th1-mediated dominant profile, and the latter could support the existence of an association between migraine and some inflammatory or autoimmune diseases. The contribution of this systemic pro-inflammatory status in activating trigemino-vascular system and facilitating neurogenic inflammation during migraine attacks remains to be established.

Higher levels of IL-10 have been observed during migraine attacks, compared to the interictal period [72, 79, 80], but, after the administration of antimigraine drug sumatriptan, IL-10 plasma levels decrease [78]. This anti-inflammatory cytokine plays a key role in immune regulation and in limiting autoimmune disease progression [81, 82]. However, an increase of IL-10 levels during attack, rather than being an expression of a so-called ‘Th2-dominant immune response’ in migraine (as that observed in atopic and allergic diseases), might be interpreted as a compensatory mechanism involved in antagonizing pro-inflammatory cytokines during the ictal period, by exerting anti-nociceptive effects and limiting neurogenic inflammation.

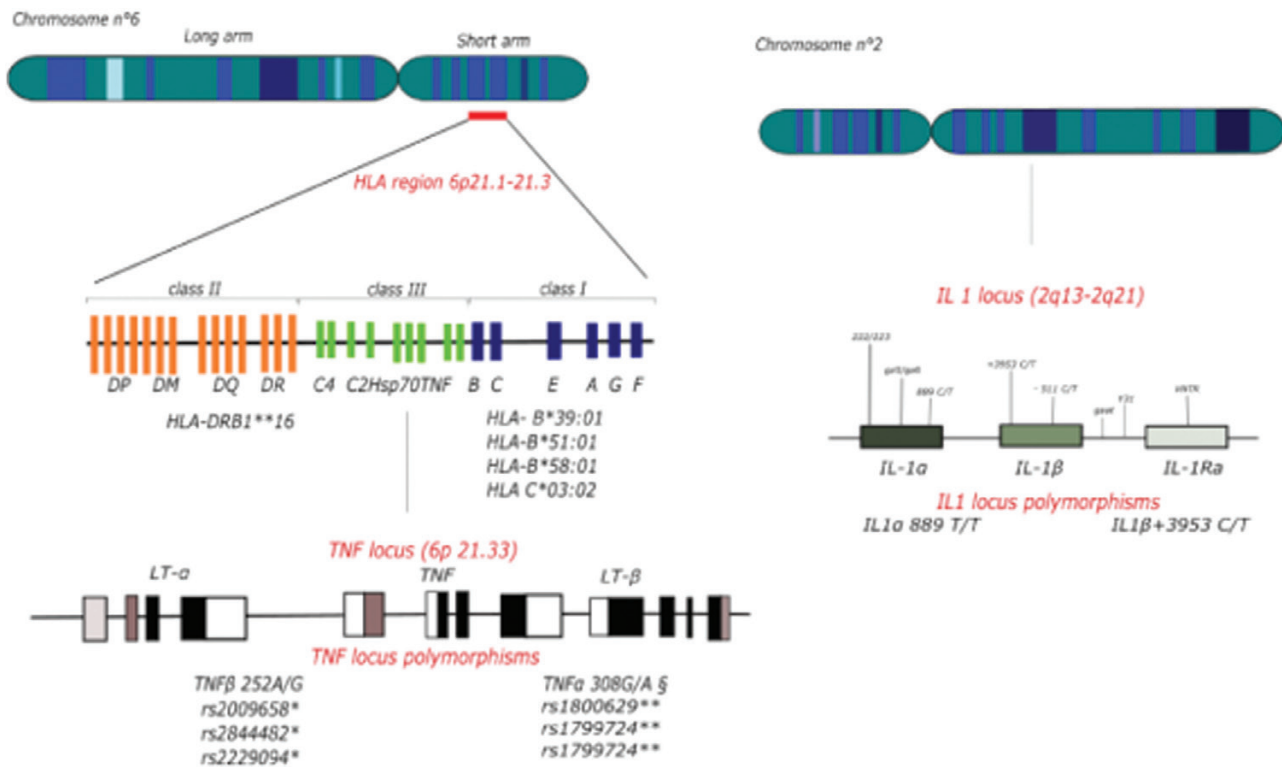


Fig. 2. Cytokine polymorphisms associated with the risk for migraine and migraine clinical presentation. Cytokine polymorphisms investigation in migraineurs mainly cover some TNF and IL1 variants. The main results of available studies are summarized. IL1, interleukin 1; LTA, lymphotoxin; MHC, major histocompatibility complex; GWAS, genome-wide association study; TNF, tumour necrosis factor.

The antinociceptive effect of IL-10 has been observed in several experimental pain models, also through the induction of β -endorphin expression [83, 84]. Moreover, IL-27 has been reported to antagonize neuropathic pain via an increase in IL-10 production. The relationship between IL-10 and IL-27 peripheral levels has never been investigated in primary headaches [85]. Conversely, a Th2-dominant response in some migraine patients could be actually hypothesized to explain an association between headache and allergic disorders and asthma, but unfortunately, to date, the cytokine profiles including plasma levels of IL-10 have never been investigated in migraine patients affected also by allergic disorders and asthma. (More details on cytokine and chemokine changes in migraineurs assessed in the interictal or/and ictal period are shown in Table 1).

Some studies have investigated pro-inflammatory molecules concentrations in jugular blood as direct consequences of trigemino-vascular activation. Specifically, a study by Sarchielli *et al.* in 2006 [87] measured pro-inflammatory cytokines concentrations and the expression of adhesion molecules on lymphocytes in migraine patients during attacks. The authors found, in the internal jugular blood, a transient increase of TNF- α and soluble intercellular adhesion molecule-1 (sICAM-1) levels at the beginning of attacks, while a subsequent progressive decrease during the attack. Moreover, this study reported a down-expression of the integrin lymphocyte function-associated antigen-1 (LFA-1) at 2 and 4 hours after attack onset. According to the authors, the increases in TNF- α and sICAM-1 immediately after the attack onset support the hypothesis of a transient neuroinflammation in migraine pathophysiology. Conversely,

the subsequent progressive decrease in sICAM-1 levels observed over the course of the attacks as well as the down-expression of LFA-1 on lymphocytes some hours after the onset could be interpreted as immunological mechanisms antagonizing the lymphocytes transvascular migration. Other findings in favour of a transient neuroinflammation during migraine attacks include (i) an increase in the jugular blood in nuclear factor-kB (NF-kB) activity peaking 2 h after attack onset and (ii) the up-regulation of nitric oxide synthase inducible isoform (iNOS) in monocytes, evident at 4 h from onset, maintained up until 6 hours from attack onset and reduced at the end of the attack. A further study by the same group evidenced a transient increase in the IL-8 levels during attacks, in internal jugular venous blood of MWA patients. IL-8 increase went hand in hand with a rise in CGRP levels. These results are in agreement with experimental findings for CGRP-induced activation of IL-8 gene expression, via the transcriptional factor AP-2, which mediates transduction in response to cyclic adenosine monophosphate [88]. Despite the evidence that chemokine IL-8 is transiently increased during migraine attacks, an accumulation of leukocytes secondary to neurogenic inflammation is unlikely, as this increase is self-limiting, as in other inflammatory events.

Taheri *et al.* [89] compared the expressions of RNA-coding genes of IL-2, IL-4, CXCL8, IL-17, IFN- γ , TGF- β , and TNF- α cytokines in blood specimens of patients with migraine and those of healthy controls in order to identify any possible dysregulation. The authors reported that the expressions of RNA-coding genes of INF- γ , IL-4, TGF- β , and TNF- α were significantly increased from a statistical point of view in migraine cases, especially in males.

Table 1: Findings on peripheral levels of cytokines and chemokines in migraineurs

Authors and year of publication (ref.)	Patients age range/mean age (years) F/M	Controls Age range/mean age (years) F/M	Headache Diagnostic criteria	Cytokine assessed	Method	Time of assessment	Main results	Comments
Martelletti <i>et al</i> 1997 [77]	20 MwA pts mean age: 36.5 ± 5.1 years 13 F and 7 M	20 healthy subjects mean age: 33.7 ± 8.4 years 13 F and 7 M 10 episodic TTH pts mean age: 37.0 ± 4.1 years 4 males and 6 females	IHS Criteria (1st edition)	serum IL-4 Other variables assessed: ICAM-1 expression IL-1R sICAM-1	ELISA method flow cytometry	Spontaneous attacks M crisis experimentally induced by the administration of isosorbide dinitrate	A sharp decrease in the expression of ICAM-1, sICAM-1 and serum IL-4 were observed in experimentally induced and spontaneous M attacks. No change of IL-1R expression values.	M patients are more sensitive to exogenous NO than C. Experimental M crisis, induced by a NO donor, is mediated by the inhibition of IL-4 and subsequently of ICAM-1. ICAM-1 downregulation inhibits the critical step of transendothelial migration into the brain of activated leukocytes in line with the 'sterile inflammation' hypothesis of M..
Munno <i>et al</i> 1998 [76]	22 MwA patients Age range: 20 to 59 years (mean age: 34.2 years) M to F ratio: 9:23	32 sex- and age-matched blood donors as healthy controls	IHS Criteria (first edition)	Plasma IFN-γ, IL-4, IL-5, and IL-10	ELISAmethod	Interictal	No difference in the plasma levels of IFN-γ and IL-10 were between M patients and C. A strong increase of IL-5 level was found in 84.3% as well as increased IL-4 levels in 37.5% of pts with MwA.	These findings suggests a preferential enhancement of some Th2-type cytokines, and may support potential immune-allergic mechanism in M.
Munno <i>et al.</i> , 2001 [79]	23 MwA patients Age range: from 19 to 58 years (mean, 38.5 years) M to F ratio: 9:14	23 subjects sex- and age- matched were included as healthy controls	IHS Criteria (first edition)	Plasma IL-4, IL-5, IL-10, and IFN-γ	ELISA method	Ictal and after acute treatment	Low to undetectable IL-5 and IL-4 levels were found. High IL-10 levels were seen in 52.2% of M pts. IFN-γ levels were undetectable in all pts. After treatment with sumatriptan, 10 pts showed a decrease in IL-10 and an increase in both IL-4 and IL-5 levels.	A preferential enhancement of TH2-type cytokine production may contribute to the mechanisms of M attacks.
Perini <i>et al.</i> , 2005 [72]	25 MwA Patients (22 MwA, 4 MA, and 1 MwA and MA) Age range: 23 to 48years (mean age: 35 ± 7.2 years) 22 F and 3 M	18 healthy subjects Age range: 23 to 51 yrs (mean age: 34 ± 9.9 years) 13 F and 4 M	IHS criteria (second edition)	Plasma IL-1β, TNF-α, and IL-10	ELISAmethod	interictal and ictal	TNF-α, IL-1β and IL-10, during attacks were significantly higher in comparison to their levels outside attacks (P = 0.0003, P = 0.03, and P = 0.05, respectively). IL-10 and TNF serum levels were higher inpatients assessed soon after headache onset and lower over time.	These cytokines are suggested to be involved in M pathogenetic mechanisms.

Table 1. Continued

Authors and year of publication (ref.)	Patients age range/ mean age (years) F/M	Controls Age range/mean age (years) F/M	Headache Diagnostic criteria	Cytokine assessed	Method	Time of assessment	Main results	Comments
Boćkowski et al., 2009 [73]	21 Children Age range: 10 to 18 years (mean, 14.04 ± 2.29) 12 MwA (6 F and 6 M) 9 MA (4 F and 5 M)	24 TTH patients Age range: 8 to 17 years (mean age: 12.11 ± 3.46 years) 18 girls, 6 boys	IHS Criteria (second edition)	Plasma IL-1β, TNF-α, and TNF receptor 1	ELISA method	Interictal	Soluble TNF receptor 1 in the M group were significantly higher than in the C group. M pts tended to have increased TNF-α level, compared with C. IL-1β level was significantly higher in MA than in MwA. TNF-α, and soluble TNF receptor 1 levels tended to be increased in MA subgroup. IL-4 was detected in 17.1% of pts with M and in 28.6% of pts with TTH. IL-13 was detected in 17.1% of pts with M and in 15.2% of pts with TTH. IL-10 was only detected in 3 of 68 (4.4%) pts. No significant correlations emerged between measurable cytokine levels and age, gender, aura, duration of disease, frequency and severity of headache in both patient groups.	Proinflammatory cytokines may be involved in the pathogenic events underlying M attacks, although fluctuations in cytokine levels may be different in children than in adults. Difference could be due to long medical history of M in adult pts and frequent intake of analgesic drugs or prophylactic treatment. No changes of anti-inflammatory cytokines levels during the headache-free period, excluding their potential involvement in pathogenic mechanisms of M and TTH in children.
Boćkowski et al., 2010 [74]	35 M patients Age range: 10–18 years (mean age 14.04 ± 2.29 years) 21 MwA (9 F and 12 M) 14 MA (6 F and 8 M)	33 TTH patients Age range: 8–17 years old (mean age: 12.11 ± 3.46 years) 22 F and 11 M	IHS Criteria (second edition)	Plasma IL-4, IL-10 and IL-13	ELISA method	Interictal		
Uzar et al., 2011 [69]	64MwA and MA 25 assessed in ictal period and 39 in the interictal period mean age: 35.4 ± 11.5 years 45 F and 19 M	34 healthy subjects mean age: 34.7 ± 11.7 years 24 F and 10 M	IHS criteria (second edition)	Serum TNF-α, IL-1β, IL-2, IL-6, IL-10, and pro BNP levels	chemiluminescence assay.	Interictal and ictal	Significantly higher concentrations of IL-1β and IL-6 and conversely significantly lower IL-10 in M pts compared with the healthy C. No differences in the cytokine levels between interictal and ictal periods. M pts had higher concentrations of pro-BNP compared with healthy C.	These cytokines are proposed to be involved in neurogenic inflammation due to trigemino-vascular activation in M, Increased pro-BNP may indicate preclinical cardiac involvement in patients with M.

Table 1. Continued

Authors and year of publication (ref.)	Patients age range/mean age (years) F/M	Controls Age range/mean age (years) F/M	Headache Diagnostic criteria	Cytokine assessed	Method	Time of assessment	Main results	Comments
Duarte <i>et al.</i> 2015 [70]	49 MwA mean age: 40.5 ± 14.5 years 46 F and 3 M	49 healthy subjects mean age: 42.5 ± 14.3 years 46 F and 3 M	IIHS criteria (second edition)	Serum CXCL8/IL-8 CCL3/MIP-1α	ELISA method	Interictal	CXCL8/IL-8 and CCL3/MIP-1α levels were significantly higher among pts with M even after controlling for anxiety and depression scores.	CXCL8/IL-8 and CCL3/MIP-1α levels were raised in M, independently of psychiatric comorbidities, migraine impact, and allodynia.
Oliveira <i>et al.</i> 2017 [71]	20 MwA and/or MwA mean age 33.8 ± 10.5 years All F	17 healthy controls mean age 33.7 ± 9.0 years All F	IIHS criteria (second edition)	Plasma TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12p70	ELISA method	Interictal	TNF-α and IL-12p70 were significantly higher, while IL-6 ($P < 0.01$), IL-8 ($P < 0.01$), and IL-10 ($P < 0.01$) were decreased compared to C group. M was positively associated with TNF-α and IL-12p70, and negatively associated with IL-6, IL-8, and IL-10. Anxiety scores were positively associated with IL-12p70. VO_{2Peak} was negatively associated with TNF-α.	An exaggeratedly skewed cytokine profile, in particular the TNF-α and IL-12p70/IL-10 balance may be related to M pathophysiological mechanisms, and its psychiatric comorbidities and functional capacity.
Bougea <i>et al.</i> 2020 [86]	30 MwA pts Age range: 18–60 years 30 TTH pts Age range: 18–60 years Sex distribution not specified	30 healthy subjects Age range: 18–50 years Sex distribution not specified	HIS criteria, (third edition, beta version)	Salivary CRP, IL-1β and IL-6	ELISA Method	Interictal	No significant differences were found in time variation of CRP, IL-1β, and IL-6 levels between M and TTH pts. IL-1β had the highest discriminative value followed by CRP and IL-6 in separating pts (M+TTH) and healthy C. CRP and IL-6 were negatively correlated with HAM-A and BDI scores.	L1-β had the highest discriminative value between headache pts and controls compared with CRP and IL-6. CRP and IL-6 were correlated with lower symptom scores of anxiety and depression prior or immediately after the headache period in both patient groups.

BDI, Beck Depression Inventory; CRP, C reactive protein; C, controls; HAM-A, Hamilton Anxiety Rating Scale; ICAM-1, intercellular adhesion molecule; IFN-γ, interferon-gamma; IL, interleukin; Interleukin 1-Receptor, IL-1R; M, migraine; MA, migraine with aura; MwA, migraine without aura; Pro-BNP, pro-brain natriuretic peptide; pts, patients; s ICAM-1, soluble intercellular adhesion molecule 1; TNF, tumour necrosis factor; TTH, tension-type headache

With regard to cytokine levels in the CSF of patients with migraine a study by Rozen and Sweden, reported an increase in CSF TNF- α levels for all patients with chronic migraine (16/16) as well as in 95% of those patients with new daily persistent headache (19/20). These results suggest the involvement of this pro-inflammatory cytokine in the maintenance of headache in both disorders. In most patients with high CSF levels, serum TNF- α levels were normal. Since most of the positive-tested patients showed scarce or no improvement, even during aggressive inpatient treatment, the persistent rise in CSF TNF- α levels, regardless of therapy, was interpreted by the authors to be one of the causes of treatment refractoriness [90]. However, the lack of reference values for TNF- α in the CSF obtained from normal subjects did not allow to determine with reliability any clinical impact for this specific biomarker [91].

Findings on the effects of onabotulinumtoxin A (onabotA) have supported the potential role of neuroinflammation in the pathophysiology of chronic migraine. Current guidelines recommended this drug as a prophylactic therapy for chronic migraine patients refractory to other preventive treatments. Its mechanism of action is not completely understood, although studies have suggested that this involves the blockage of inflammatory neuropeptides released by stimulated trigeminal neurons [92]. Whereas data on the effect of OnabotA on neurogenic inflammation are contradictory in animal models, results from clinical studies clearly support an anti-inflammatory action of this treatment [93–95]. Furthermore, patients with higher interictal pre-treatment concentrations of CGRP along with systemic acute-phase proteins, such as pentraxin 3, seem to better respond to this type of treatment [96]. Finally, investigating the hypothesis of a link between chronic migraine and periodontitis [97], Leira and colleagues found that a mild systemic inflammation induced by periodontitis could enhance treatment response to onabotA. The authors also reported that in presence of elevated systemic inflammatory markers related to periodontitis, OnabotA might reduce the frequency migraine attacks [98]. These preliminary findings need to be replicated by further studies.

In conclusion, several studies have reported significant increases for pro-inflammatory molecules, TNF- α , IL-1 β , IL-6, and IL-8 in peripheral blood samples from migraine patients compared to controls, both in ictal and interictal periods. Available data on CSF also support the role of inflammation in migraine, but studies on CSF in migraineurs have been few and mostly dealing with chronic migraine.

Data available on cytokines in migraineurs therefore seem to suggest a prevailing pro-inflammatory systemic status in migraineurs evident in interictal phase, and more accentuated during attacks. More specifically, a role of some note might be played by TNF- α which has been elevated not only in the peripheral blood of migraineurs, but also in the CSF of patients affected by migraine chronic form.

Changes in cytokines and other leading immune mediators during attacks from jugular blood also suggest a transient local activation of pro-inflammatory events over the course of neurogenic inflammation, which is self-limiting and can not induce the transvascular migration of immune cells into the brain, as in autoimmune or inflammatory diseases involving cerebral parenchyma. The possible relationship between the systemic pro-inflammatory status and local immune activation in the trigeminal pathway is unknown. It might be plausible that systemic pro-inflammatory status

fosters the trigemino-vascular activation at dural and pial vessels, leading to migraine attack onset.

Immune cell subsets in migraineurs

While the transient immunological changes during attacks have been suggested to be related to trigemino-vascular system activation, alterations of specific immune parameters in the peripheral blood of migraineurs assessed during the interictal period suggest a more general immune dysregulation which could predispose patients to immunological and autoimmune disorders. Alterations of lymphocyte subsets have been observed in migraine patients [7, 68, 99, 100]. These include a significant increase in the CD4+ and a decrease in the CD8+ lymphocyte subsets in migraine patients [101, 102]. Additionally, an increase in the percentage of the CD3+CD16+CD56+ lymphocyte subset in migraineurs has been shown to distinguish migraineurs assessed interictally from controls [103]. The increase in this subset, commonly referred to as a NK cell subtype, has been regarded as a compensatory mechanism by contrasting the reduction of CD4+ T-helper cells [104]. This increase has also been associated to the increase of NOS activity and nitrite accumulation, which are both relevant in migraine pathophysiology [105]. Noteworthy, various triptan-like molecules have been shown to induce the inhibition of NK activity and the decrease in neutrophil metalloproteases-9 secretion. It remains to be established if this anti-inflammatory effect might be part of the mechanism of action of these specific drugs for the acute treatment of migraine [106].

The aforementioned findings regarding alterations on lymphocyte subsets support the presence of an underlying derangement of the immune system in migraine patients. This derangement might favour the triggering of some immunological and autoimmune disorders in migraineurs. In fact, evidence of an impairment of CD8+ cells and an increase in CD4+ cells have been observed in several autoimmune diseases, as they do in migraine [107, 108].

A study by Arumugam and Parthasarathy investigated T-cell subsets placing particular interest on regulatory T (Treg) cells in migraine patients [109]. This lymphocyte subset, which specifically expresses CD4 and CD25 molecules on the surface and the transcription factor forkhead box protein 3 (FOXP3), regulates the local immune response through cell-to-cell contact and the production of anti-inflammatory cytokines including IL-9, IL-10, and TGF- β 1 [110, 111], which in turn inhibits CD4+ cell proliferation [112]. Therein, CD4+ CD25+ lymphocytes play a pivotal role in the prevention of autoimmunity [113–115], by suppressing altered immunological responses [113, 116]. In the Arumugam and Parthasarathy's study, in line with previous studies, a significant increase in CD4+ and a decrease in CD8+ lymphocyte subsets were recorded in migraine patients, compared to healthy controls. The authors also reported that immunoregulatory CD4+CD25+ cell levels were lower in migraine patients, compared to controls [109]; credibly suggesting that the failure of self-recognition mechanisms might play a determining role in migraine pathogenesis and predispose migraineurs to immunological/autoimmune disorders (Fig. 3). Another investigation confirmed a reduction of Treg cells for MoA nad MA patients compared to controls [117]. These interesting results need to be tested and validated by studies including larger populations of migraineurs.

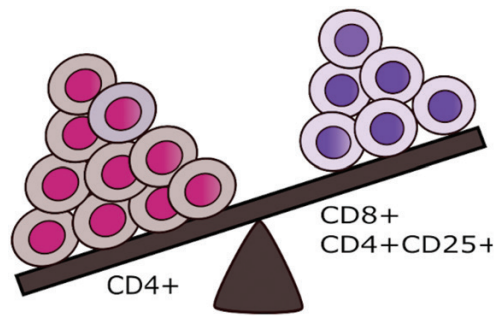


Fig. 3. The failure of self-recognition mechanisms in migraine pathogenesis. In this figure, we represent the imbalance between lymphocytes subsets in patients with migraine as compared to healthy controls (CD4 + are increased in migraineurs, while CD8 + and CD4 + CD25+ regulatory cells are reduced).

Finally, Pavelec *et al.* carried out a study assessing for any changes in peripheral blood parameters of acquired immunity in patients with migraine [118]. In this study, episodic migraine sufferers had increased values, compared to healthy subjects, for relative count of lymphocytes, relative and absolute counts of CD3+ T cells, relative and absolute counts of CD8+ suppressor/cytotoxic T cells, relative and absolute counts of CD4 + T_{EMRA} (terminally differentiated helper T lymphocytes), absolute count of CD8+ naive T cells, and absolute count of CD19+ switched memory B cells. Strikingly, CD4 + T_{EM} (effector memory helper T lymphocytes) and CD8 + T_{EMRA} appeared to be inversely associated with Headache Impact Test-6 (HIT-6) score values. More specifically, migraine patients with a CD4 + T_{EM} values below 15 had a high probability (90%) that the HIT-6 value would be higher than 60. Therefore, CD4 + T_{EM} was proposed by the authors as a biomarker for migraine severity.

Immunological findings in CH

Even for CH, limited evidence point to an underlying role of immunological dysfunctions in the pathogenesis of this disorder. Since the 1980s, studies have reported on a link between CH and genes implicated in the regulation of the immune system. One of these studies detected a lower frequency of HLA-B14 in patients with CH, compared to controls, suggesting that this HLA haplotype could represent a mechanism of resistance against this form of headache [119]. In 1987, a study by Giacovazzo *et al.* found an absolute monocytosis during the CH period, which was correlated with the class II antigen, HLA-DR5 [120]. The same research group reported a significant increase in the pro-inflammatory cytokine IL-1 β for CH patients during attacks, compared to those assessed between attacks and normal controls. Furthermore, this study reported higher levels of IL-1 β in patients with CH in the interictal period, compared to control subjects [121]. A full decade later, a study by Empl and colleagues observed a significant increase in soluble IL-2 receptors from the serum of CH patients assessed in the active period, compared to controls. The same study evidenced a trend toward an increase in IL-1 in CH patients, this did not result statistically significant [122].

Subsequent findings have bolstered the role of the immune system in CH pathogenesis, by reporting results indicating

cytokines are released during the active period of the disease. To this regard, a study by Steinberg and colleagues stated that a significant increase in IL-2 gene expression, assessed by means of real-time polymerase chain reaction, occurs between attacks during active periods of CH, but not in attack remission. As for the latter phase, the authors reported that IL-2 gene expression did not differ from that of controls [123].

A study by Goadsby and Edvinsson first reported on a significant increase in CGRP in the jugular blood of CH patients during attacks, as already had been done for migraineurs. Moreover, the ability of both sumatriptan and oxygen was shown in reducing CGRP levels [22]. A later investigation evidenced the capacity of corticosteroids to cut CGRP levels in CH patients, further supporting the pathogenic neuroinflammatory hypothesis for CH [124]. In line with these findings, a pre-clinical research involving primary cultures of rat trigeminal cell ganglia found that methylprednisolone, but not metoprolol, was able to block 1L-1 β -induced CGRP release [125]. Based on these results, the pivotal role of this neuropeptides in CH pathophysiology and neuroinflammation subsiding trigeminovascular activation during attacks was defined and it has been identified as a target for current more tailored treatments targeting CGRP [86].

In light of the limited immunological findings on CH, potential changes in the pro-inflammatory cytokines might be associated to CH, particularly in the active phase, but no conclusion on a specific cytokine pattern for this primary headache can be drawn nor can differences between CH and migraine be hypothesized.

Therefore, future research on this topic will need to include a thorough simultaneous assessment of a complete cytokine and chemokine panel, for example, with Luminex technique, in CH patients assessed in active and non-active periods. These data should be compared with those obtained in migraineurs, episodic and chronic, investigated both ictally and interictally.

Immunological findings in tension-type headache

Unlike migraine and CH, where the role of neuroinflammation via trigemino-vascular activation is quite accepted among the scientific community, the pathophysiology of TTH is not well understood. Specifically, little is known about the implication of the derangement of the immune system in this primary headache. Nevertheless, also for TTH, some studies have reported an increase for the levels of some cytokines in these patients, compared to controls. For example, a study by Domingues *et al.* in 2015 reported a significant increase in IL-8 serum levels for TTH patients, compared to age and sex-matched controls [126]. This difference persisted also after controlling for anxiety and depression, which were more prevalent in the TTH group. Other studies have evidenced a significant increase in other pro-inflammatory cytokines, including IL-6 and IL-1 β [127].

A longitudinal prospective study conducted on 30 migraineurs, 30 TTH patients, and 30 age-matched healthy controls revealed no significant differences in time variation for C-reactive protein (CRP), IL-1 β , and IL-6 salivary levels between patients with migraine and those with TTH. Moreover, IL1- β had the highest discriminative value

in separating headache patients (both TTH and migraine) and controls, compared with CRP and IL-6. In both patient groups, CRP and IL-6 correlated with lower scores for anxiety and depression prior to or immediately after the headache period [128].

Furthermore, the role of cytokines in TTH has also been suggested by a study carried out by Bø *et al.*, where CSF levels of some inflammatory and anti-inflammatory molecules were measured in a cohort of 127 patients: 34 MwA, 24 MA, 39 episodic TTH, 10 cervicogenic headache, and 20 pain-free subjects enrolled as control group. Of the seven cytokines investigated, measurable amounts of only three- IL-1 receptor antagonist (ra), monocyte chemoattractant protein-1 (MCP-1), and TGF-1-were found in the CSF for all patients. The main observed differences were between pain-free control group and TTH, and also between controls and MwA. In both headache groups, IL-1ra, MCP-1 and TGF-b1 were significantly higher compared to controls. In the case of MA, significant differences were reported only for levels of IL-1ra and MCP-1, but not of TGF-β1. Finally, there were no significant differences observed between the control and cervicogenic headache groups [129].

Ultimately, TTH data on peripheral cytokine levels are limited making it difficult to suggest any specific cytokine or chemokine profiles based on the available findings. Being so, as suggested above here for CH, a Luminex technique would be best suited in a study that sought to identify changes in blood levels of a variety of cytokines and chemokines both in episodic and chronic TTH patients, the former could be assessed both ictally and interictally.

Conclusive remarks

Experimental evidence from animal models of trigemino-vascular activation suggests a pivotal role for neurogenic inflammation involving for the most part mast-cells around meningeal vessels, as well as glial cells and astrocytes in both TG and TNC. In this setting, many pro-inflammatory cytokines, which contribute to maintain sensitization of trigeminal fibres, are produced.

With this in mind, the prevalent involvement of pro-inflammatory mediators and specifically cytokines is strongly supported by data on their peripheral blood levels from migraine patients assessed both ictally and interictally. Changes in lymphocyte subsets of migraineurs also support a derangement of the immune system which could favour, in some patients, the clinical emergence of immunological/autoimmune disorders.

The presence of a systemic pro-inflammatory status is therefore the most robust finding in migraine. In spite of the limited findings available on cytokine concentrations in CSF, it is likely that their changes express similar changes in central nervous system (CNS), especially in the structures involved in head pain processing.

Data concerning jugular blood are also limited, but seem in line with results from experimental studies showing the occurrence of neuroinflammation as a consequence of trigemino-vascular activation.

It remains to understand the contribution of the proinflammatory status in migraineurs in reducing the threshold for activation of trigeminovascular system that is believed to be responsible for fostering neuroinflammatory events during migraine attacks.

Even for CH), the involvement of neurogenic inflammation is considered to be a key mechanism involved in the induction and maintenance of attacks. Therefore, pro-inflammatory mediators are thought to be involved in its pathogenesis. Results from studies on immunocompetent cells and pro-inflammatory cytokines in CH patients, suggest a potential implication of pro-inflammatory activity, at least during active phases of the disease.

In addition, results concerning the involvement of inflammatory mediators in TTH pathophysiology are less conclusive, although alterations in some immunological parameters have been observed. The main immunological findings for primary headaches are summarized in Table 2.

Therefore, based on the current literature, a role for the immune system can be affirmed for migraineurs and perhaps also for CH patients and this can have significant therapeutic implications. As migraine patients with refractory headaches, history of recurrent headaches, severe baseline disability, and status migrainous have the most benefit from corticosteroid therapy, this further supports the involvement of neuroinflammation resulting from trigemino-vascular activation [130]. Equally important, the proven efficacy of short-term steroid treatment in reducing frequency of cluster attacks clearly supports a role of neurogenic inflammation and inflammatory mediators also in this primary headache [131–134].

In conclusion, a significant role of neuroinflammation in primary headaches seems to be plausible, but, in order to definitely clarify this issue, further studies should be performed in the next years mainly in non-migraine primary headaches field. Specifically, in our opinion, large studies exploring CSF and blood levels of pro-inflammatory molecules in CH and tension-type headache and their eventual correlation with specific polymorphisms of genes encoding pain and inflammation mediators could be of interest. Furthermore, randomized controlled trials aiming to evaluate the effect of targeted and untargeted anti-inflammatory drugs simultaneously on both clinical and biochemical parameters of pain and inflammation in the context of primary headaches could very useful in the view of a deeper comprehension and a better management of these complex and often disabling disorders.

Conflict of interest

None declared.

Author contributions

L.B. and P.S. wrote the paper. G.D.V., E.C., A.B., M.L.C., and P.C. searched data and revised the manuscript. All the authors approved the final manuscript.

Data availability

All data reported in this review can be found in the papers cited.

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Table 2 : Summary of main immunological findings in primary headache patients Migraine

Cytokines/Chemokines/adhesion molecules	Plasma Peripheral blood	Plasma Peripheral blood	Plasma Jugular blood	CSF
	Interictal period *	Ictal period**	Ictal period **	Interictal period *
IL-12	↑			
IL-1β	↑	↑↑		
IL1 ra				↑
TNF-α	↑	↑↑	↑	↑
IFN-γ	↑	↑↑		
IL-2		=		
IL-6	↑	↑↑		
IL-4	↓	↓		
IL-5	↓	↓		
TGF-β	↑			↑
IL-10	↓	↑		
IL-8	↑		↑	
CCL3/MIP-1α	↑			↑
ICAM-1			↑	
Cluster headache				
	Plasma Peripheral blood	Plasma Peripheral blood		
Cytokines	Interictal period *	Ictal period**		
IL-1β	↑	↑↑		
IL-2 r (soluble)	↑ §			
Tension type headache				
	Plasma	CSF	Saliva	
Cytokines/Chemokines	Interictal period *	Interictal period *	Interictal period *	
IL-1β	↑		↑↑	
IL1 ra		↑		
IL-6	↑		↑	
TGF-β		↑		
IL-8	↑			
CCL3/MIP-1α		↑		

*vs. control subjects; **vs. interictal period.
 †increase ††further increase ‡decrease = no changes § active phase.

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