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The Tachykinins Substance P and Hemokinin-1 Favor the Generation of Human Memory Th17 Cells by Inducing IL-1β, IL-23, and TNF-Like 1A Expression by Monocytes

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The nervous system influences immune responses through the release of neural factors such as neuropeptides. Among them, the tachykinin substance P (SP) signals via the neurokinin 1 receptor (NK-1R), which is expressed by various immune cells. We thereby analyzed in this paper whether tachykinins may participate in human CD4$^+$ Th cell polarization. We report that SP and hemokinin-1 (HK-1) upregulate IL-17A and IFN-γ production by human memory CD4$^+$ T cells without affecting IL-4 and IL-10 production. SP and HK-1 switch non–Th17-committed CD4$^+$ memory T cells into bona fide Th17 cells and Th1/Th17 cells. In contrast, SP and HK-1 do not modulate the polarization of naive CD4$^+$ T cells. SP- and HK-1-induced Th17 cell generation is mediated through NK-1R and requires the presence of monocytes. SP and HK-1 trigger IL-1β, IL-6, and TNF-α production, upregulate IL-23 production, and enhance TNF-like 1A expression on monocyte surface. Neutralization experiments demonstrated that IL-1β, IL-23, and TNF-like 1A are involved in the SP- and HK-1–induced Th17 cell. The other members of the tachykinin family, neurokinins A and B, have no effect on the differentiation of naive and memory T cells. These results thereby show that SP and HK-1 are novel Th17 cell-inducing factors that may act locally on memory T cells to amplify inflammatory responses. The Journal of Immunology, 2011, 186: 4175–4182.

Neuropeptides are nerve-secreted peptides that mediate or modulate neuronal communications and influence brain functions, including memory, learning, analgesia, and food intake. Some neuropeptides also influence innate and adaptive immunity by interacting with immune cells in the lymphoid organs (via the autonomic nervous system), in the bloodstream (via the neuroendocrine system), or at inflammatory sites (via sensory peripheral nerves) (1, 2). Tachykinins are 10–11 aa neuropeptides that share a common C-terminal pentapeptide essential for their biological activities. This family includes four members: substance P (SP), neurokinin A (NKA), neurokinin B (NKB), and the recently identified hemokinin 1 (HK-1) (3).

In the CNS, SP is involved in behavior, stress, cognition, and vomiting reflex (3, 4). SP is also released by afferent neurons innervating peripheral tissues and is involved in pain perception, vasodilation, vascular leakiness, and edema (5). In addition to neurokinins A and B, no effect on the differentiation of naive and memory T cells. These results thereby show that SP and HK-1 are novel Th17 cell-inducing factors that may act locally on memory T cells to amplify inflammatory responses. The Journal of Immunology, 2011, 186: 4175–4182.

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Abbreviations used in this article: DC, dendritic cell; HK-1, hemokinin-1; IBD, inflammatory bowel disease; NKA, neurokinin A; NKB, neurokinin B; NK-1R, neurokinin receptor 1; PFA, paraformaldehyde; PGN, peptidoglycan; qPCR, quantitative PCR; RA, rheumatoid arthritis; SP, substance P; TL1A, TNF-like 1A; Treg, regulatory T.

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subset differentiation is tightly controlled by environmental factors and especially by cytokines (e.g., IL-4 and IL-12 that contribute to Th2 and Th1 polarization, respectively). Some neuropeptides also modulate Th cell differentiation (1, 2, 21). As examples, Bradykinin induces IL-12–producing DCs and favors Th1 polarization (22), whereas calcitonin gene-related peptide polarizes Langerhans cells to promote a Th2-type immunity (23).

An enhanced expression of SP has been reported in inflammatory sites such as colonic mucosa of patients with inflammatory bowel disease (IBD), rheumatoid arthritis (RA) synovial fluids, and in psoriatic skin (9, 24, 25). Because immune cells are sensitive to tachykinins (9, 10), we analyzed in this study whether tachykinins may regulate human Th cell polarization. Results showed that SP and HK-1 favor the generation of human memory Th17 cells by inducing IL-1β, IL-23, and TLLA expression by monocytes.

Materials and Methods
Cytokines and tachykinins
Recombinant human IL-1β (Milleniy Biotec, Bergish Gladbach, Germany), IL-2, IL-6 (ImmunoTools, Friesoythe, Germany), and GM-CSF (CellGenix, Freiburg, Germany) were purchased from the indicated providers. SP, HK-1, NKA, and NKB were from Sigma-Aldrich (St. Louis, MO).

Cell purification
Blood from healthy subjects were obtained in accordance with the guidelines of the Angers University Hospital ethics committee. PBMCs were isolated from blood by density centrifugation with lymphoprep separation medium (NH4Cys, Paris, France), and CD14+ monocytes were purified from PBMCs by positive selection (Miltenyi Biotec); purity was >99% (data not shown). After depletion of non-CD4+ T cells from PBMCs, CD4+CD45RO− naive T cells and CD4+CD45RO+ memory T cells were purified by negative and positive selection, respectively (Milltenyi Biotec); purity was >98% (data not shown). IL23R+ memory non-Th17 cells (26) were isolated from memory CD4+ T cells by FACS using allophycocyanin-labeled anti-CD4, FITC-labeled anti-CD45RO (both from BD Pharmingen, San Diego, CA), and PE-labeled anti-IL-23R mAbs (R&D Systems, Abingdon, U.K.); purity was >99% (data not shown).

Cell stimulation
A total of 10^5 naive or memory CD4+ T cells, cultured in X-VIVO 20 medium (Lonza, Verviers, Belgium), were stimulated with immobilized anti-CD3 mAb (OKT3) in the presence of 5 × 10^5 autologous monocytes or 1 μg/ml anti-CD28 mAb (BD Pharmingen). SP, HK-1, NKA, or NKB were added at the indicated concentrations. IL-1β (10 ng/ml) and IL-6 (20 ng/ml) or TNF-α (all from R&D Systems), TL1A (BioLegend, San Diego, CA), or isotype-matched mAbs (from R&D Systems and BioLegend). In others, monocytes and T cells were cultured separately using Transwell inserts (0.4 μm pore size) (Corning Costar, Cambridge, MA), or T cells were cultured with monocytes fixed with 1% paraformaldehyde (PFA) (Euromedex, Mundolsheim, France) (30).

Cytokine quantification
IL-1β, IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF-α ELISA were from Diaclone (Besançon, France). IL-23 ELISA was from eBioscience (San Diego, CA).

Intracellular cytokine staining
Memory CD4+ T cells were stimulated with immobilized anti-CD3 mAb, in the presence of monocytes, without or with SP or HK-1. IL-2 (5 U/ml) was added at day 7. After 7 additional days of culture, memory CD4+ T cells were stimulated with 10 ng/ml PMA and 1 μM ionomycin for 6 h in the presence of 10 μg/ml brefeldin A (all from Sigma-Aldrich). T cells were then fixed with 4% PFA and incubated for 30 min in PBS containing 0.1% BSA and 0.1% saponin (both from Sigma-Aldrich) with FITC-labeled anti–IFN-γ mAb (BD Pharmingen) and PE-labeled anti–IL-17A mAb (eBioscience). Fluorescence was analyzed by cytofluorometry (FACScan; BD Biosciences, Erembodegem, Belgium).

Western blotting
Proteins (corresponding to 5 × 10^5 cells) were electrophoretically separated on a 10% polyacrylamide gel in reducing conditions and transferred to a membrane (Immobilon; Millipore, Billerica, MA). After saturation, membranes were incubated overnight at 4°C with 2 μg/ml anti-TL1A mAb (BioLegend) and 1 h with 1 μg/ml peroxidase-labeled anti-mouse IgG Ab (BioSource International, Camarillo, CA). Protein loading was verified with an anti–β-actin polyclonal Ab (Sigma-Aldrich) revealed by a peroxidase-labeled anti-rabbit polyclonal Ab (BioSource International). Bound Abs were detected using the ECL system (GE Healthcare, Buckinghamshire, U.K.).

PCR analysis
Monocytes were cultured for 6 h, as described previously. Total RNA was extracted using the RNeasy Plus MiniKit (Qiagen, Düsseldorf, Germany) and reverse transcribed using the superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). RNA integrity and cDNA synthesis were verified by amplifying GAPDH cDNA. For RT-quantitative PCR (qPCR), amplification was done using IQ SYBR Green Supermix (Bio-Rad, Hercules, CA), and specific gene expression was calculated using the 2^{-ΔΔCT} method (31) (using GAPDH as calibrator). The primer sequences used are the levels of cytokine production (mean ± SEM) determined as follows: 1 – [(B − A)/(B − A)] × 100, where A and B are the levels of cytokine produced without and with tachykinins, respectively, in the presence or not of antagonists. Data were analyzed by the two-tailed Wilcoxon matched pairs test, with p < 0.05 being considered significant.

Statistical analysis
Data are shown as cytokine levels (mean ± SEM) or as a percentage of inhibition of cytokine production (mean ± SEM) determined as follows: 1 – [(B − A)/(B − A)] × 100, where A and B are the levels of cytokine produced without and with tachykinins, respectively, in the presence or not of antagonists. Data were analyzed by the two-tailed Wilcoxon matched pairs test, with p < 0.05 being considered significant.

Results
SP and HK-1 potentiate the generation of human memory Th17 cells
To evaluate whether tachykinins may modulate CD4+ T cell polarization, highly pure naive and memory CD4+ T cells were stimulated with an anti-CD3 mAb and cultured with autologous monocytes in the absence or presence of SP, HK-1, NKA, or NKB. IFN-γ, IL-4, IL-10, and IL-17A were quantified to monitor Th1, Th2, Treg, and Th17 cells, respectively. Results showed that SP and HK-1 significantly upregulated IL-17A production by memory T cells (percentages of increase of 218 ± 37 and 246 ± 32%, respectively; mean ± SEM, n = 8), to a similar extent than PGN (percentage of increase of 218 ± 56%), used as a positive control of IL-17A and IFN-γ production (Fig. 1A, left panel) (27, 29). The effect of SP and HK-1 was dose dependent, significant at 1 μM, and maximal at 10 μM, the highest concentration used (Fig. 1B). In contrast, NKA and NKB did not modulate IFN-γ, IL-4, IL-10, and IL-17A secretion by memory T cells, whatever the concentration used (Fig. 1B; data not shown). SP and HK-1 also significantly increased IFN-γ production by memory T cells (percentages of increase of 82 ± 19 and 72 ± 5%, respectively; mean ± SEM, n = 8), although to a lower extent than PGN (percentages of increase of 146 ± 25%) (Fig. 1A, right panel), whereas IL-4 and IL-10 production was not affected (data not shown). SP and HK-1 failed to modulate IL-17 and IFN-γ production by memory T cells in the absence of anti-CD3 mAb (data not shown). Finally, none of the tachykinins induced IL-4 and IL-17A or modulated IFN-γ and IL-10 production by naive CD4+ T cells (Fig. 1A; data not shown).
We then analyzed by flow cytometry whether the increase in IFN-γ and IL-17A production induced by SP and HK-1 resulted from an increase in the frequency of Th1 and Th17 cells. Results showed that SP and HK-1 enhanced the frequency of IL-17A–producing CD4+ memory T cells (percentages of increase of 116 ± 17 and 109 ± 25%, respectively; mean ± SEM, n = 4) (Fig. 1C, right panel). Memory CD4+ T cells, stimulated for 7 d as described above, were maintained in culture with 5 U/ml IL-2 for 7 additional days. Intracellular expression of IL-17A and IFN-γ was analyzed by FACS after restimulation by PMA and ionomycin. Left panel, Dot plots are representative of one of four experiments; right panel, results are expressed as a percentage of IL-17– or IFN-γ–expressing cells (mean ± SEM, n = 4). *p < 0.05.

We then investigated whether the production of IL-17A induced by SP- and HK-1–stimulated memory CD4+ T cells resulted from a polarization of non–Th17-committed memory CD4+ T cells into Th17 cells and/or from an expansion of pre-existing memory Th17 cells. To evaluate these hypotheses, we first analyzed the ability of SP and HK-1 to induce IL-17A–producing CD4+ memory T cells (percentages of increase of 116 ± 17 and 109 ± 25%, respectively; mean ± SEM, n = 4) (Fig. 1C, right panel) and gave rise to two subsets of Th17 cells, producing either IL-17A alone (bona fide Th17 cells) or IL-17A plus IFN-γ (Th1/Th17 cells) (Fig. 1C, left panel). In contrast, the percentage of Th1 cells (IFNγ+IL-17−) was not modulated (Fig. 1C), showing that SP and HK-1 selectively upregulate the frequency of Th17 cells.

Finally, the selective nonpeptide NK-1R antagonist L703,606 (32) prevented SP- and HK-1–induced IL-17A (inhibition of 95 ± 5 and 77 ± 12%, respectively; mean ± SEM, n = 4) and IFN-γ production (inhibition of 70 ± 17 and 75 ± 15%, respectively), whereas the PGN-induced IL-17A and IFN-γ production was unaffected (Fig. 1D). In contrast, the NK-2R antagonist GR 159897 and the NK-3R antagonist SB 218795 did not significantly inhibited the SP-, HK-1–, and PGN-induced IL-17A and IFN-γ production (Fig. 1D). Collectively, these results demonstrate that SP and HK-1 increase Th17 cell generation by acting selectively via NK-1R.

**SP and HK-1 switch non–Th17-polarized CD4+ memory T cells into Th17 cells**

We then investigated whether the production of IL-17A induced by SP- and HK-1–stimulated memory CD4+ T cells resulted from a polarization of non–Th17-committed memory CD4+ T cells into Th17 cells and/or from an expansion of pre-existing memory Th17 cells. To evaluate these hypotheses, we first analyzed the ability of SP and HK-1 to induce IL-17A production by an IL-23R− memory CD4+ T cell subset (26). FACS-sorted IL-23R− and IL-23R+ memory T cells (purity > 99%) were stimulated with an anti-CD3 mAb. IL-23R−, but not IL-23R+, memory T cells produced IL-17A (Supplemental Fig. 1D; data not shown).
demonstrating that the IL-23R− population represents the non-Th17-committed memory CD4+ cell subset, as reported previously (26). IL-23R− memory T cells were then stimulated with an anti-CD3 mAb, in the presence of monocytes, without or with SP or HK-1. Results showed that SP and HK-1 potentiated IL-17A production by FACS-sorted IL-23R− memory T cells (increases of 194 ± 37 and 207 ± 36%, respectively; mean ± SEM, n = 5) (Fig. 2A). FACS analysis showed that SP and HK-1 increased the frequency of IL-17A–expressing cells in the FACS-sorted IL-23R− memory T cell population (Fig. 2A). Moreover, SP- and HK-1 increased the expression of the mRNA encoding the Th17 cells markers IL-17A, IL-23R, and RORc (Fig. 2B). Similar results were obtained with PGN, which was used as a positive control (Fig. 2B).

In a second set of experiments, we observed that SP and HK-1 did not upregulate IL-17A production and the proliferation of Th17-committed human CD4+ Th17 clones, stimulated with an anti-CD3 mAb in the presence of monocytes (Supplemental Fig. 2), thereby suggesting that SP and HK-1 do not affect IL-17 production by established Th17 cells. Collectively, these results demonstrate that SP and HK-1 polarize noncommitted memory CD4+ T cells into Th17 cells.

**SP and HK-1 promote Th17 generation through monocyte activation**

We next analyzed the mechanism(s) involved in SP- and HK-1–induced Th17 cell generation. In the absence of monocytes, SP and HK-1 failed to upregulate IL-17A production by anti-CD3 and -CD28 mAbs-stimulated memory CD4+ T cells (Fig. 3). In contrast, IL-1β plus IL-6 induced IL-17A production by CD4+ T cells (Fig. 3), as reported previously (27). This result shows that the ability of SP and HK-1 to promote Th17 cell generation is dependent on monocytes.

We then evaluated whether monocyte-derived soluble mediators and/or T cell-monocyte contacts were involved in the tachykinin-induced Th17 cell generation. Monocytes were cultured with anti-CD3 and -CD28 mAbs-stimulated memory CD4+ T cells in separate chambers (Transwell assay). In this condition, SP and HK-1 failed to upregulate IL-17A production by T cells in contrast to PGN (Fig. 3). Moreover, addition of 2-d supernatants of SP- and HK-1–stimulated monocytes to anti-CD3 and -CD28 mAbs-stimulated memory CD4+ T cells failed to upregulate IL-17A production (Fig. 3). These results suggest that T cell-monocyte contacts are involved in SP- and HK-1–induced Th17 cell generation.

We therefore investigated the role of T cell-monocyte contacts in the tachykinin-induced Th17 cell generation. Anti-CD3 mAb-stimulated memory CD4+ T cells were cultured with monocytes previously stimulated for 2 d with SP and HK-1 prior fixation with PFA. PFA-fixed monocytes previously stimulated with SP and HK-1 also failed to upregulate IL-17A production by T cells (Fig. 3). In contrast, the presence of both SP- or HK-1–stimulated monocyte supernatants and PFA-fixed monocytes previously stimulated with SP or HK-1 increased the production of IL-17A (Fig. 3). This production was similar to the one observed when activated memory T cells were stimulated with an anti-CD3 mAb in the presence of monocytes plus SP or HK-1 (Fig. 3). Collectively, these data suggest that SP and HK-1 induce the production of soluble molecules and the expression of membrane molecules by monocytes that are both involved in SP- and HK-1–induced Th17 cell generation.

**SP- and HK-1–stimulated monocytes potentiate Th17 cell generation through IL-1β, IL-23, and TLI A**

Previous studies have underlined the pivotal role of proinflammatory cytokines, including IL-1β, IL-6, IL-23, and TNF-α, in the generation and maintenance of Th17 cells (27, 33, 34). We then analyzed whether SP and HK-1 may affect proinflammatory cytokine production by monocytes. Results showed that SP- and HK-1–induced IL-1β, IL-6, and TNF-α and upregulated IL-23 production by monocytes, although to a lower extent than PGN (Fig. 4A). We thus evaluated, in neutralization experiments, the potential involvement of these cytokines in SP- and HK-1–induced IL-17A production. Memory CD4+ T cells, stimulated with an anti-CD3 mAb in the presence of monocytes, were exposed to SP or HK-1 in the absence or presence of neutralizing anti-IL-1β, -IL-6R, -IL-23, or -TNF-α mAbs. Results showed that the anti-IL-1β and anti–IL-23 mAbs inhibited IL-17A production induced by SP (inhibition of 83 ± 10 and 72 ± 2%, respectively; mean ± SEM; n = 6) and HK-1 (inhibition of 84 ± 16 and 73 ± 4%, respectively), whereas, in contrast, the anti–IL-6R and -TNF-α mAbs had no significant effect (Fig. 4B, upper panel). These results show that IL-1β and IL-23 are involved in SP- and HK-1–induced Th17 cell generation.

**FIGURE 2.** SP and HK-1 switch non–Th17-polarized CD4+ memory T cells into Th17 cells. A, IL-23R− memory CD4+ T cells were stimulated by an anti-CD3 mAb in the presence of autologous monocytes, without or with 10 μM SP, 10 μM HK-1 or 5 μg/ml PGN. Left panel, IL-17A was quantified by ELISA in the 7-d supernatants. Results are expressed in nanograms per milliliter (mean ± SEM, n = 5). *p < 0.05. Right panel, IL-23R− memory CD4+ T cells were maintained in culture with 5 U/ml IL-2 for 7 additional days. Intracellular expression of IL-17A and IFN-γ was analyzed by FACS after restimulation by PMA and ionomycin. Dot plots are representative of one of four experiments. B, IL-23R− memory CD4+ T cells were stimulated by an anti-CD3 mAb in the presence of autologous monocytes, without or with 10 μM SP, 10 μM HK-1, or 5 μg/ml PGN. After 3 d, memory T cells were isolated by magnetic sorting (negative selection), and the expression of the mRNA encoding IL-17A, IL-23R, and RORc was analyzed by qPCR. Results are expressed as the fold increase (mean ± SEM, n = 4) of mRNA expression in IL-23R− memory T cells cultured in the presence of non-stimulated monocytes; dotted line, mean ± SEM (n = 4).
We next aimed at identifying the membrane molecules involved in SP- and HK-1–induced Th17 cells. Interactions between some members of the B7 and TNF superfamilies with their receptors on T cells modulate and fine-tune CD4+ T cell differentiation (35, 36). We therefore investigated whether SP and HK-1 may modulate the expression of these molecules on monocytes. Results from qPCR showed that SP and HK-1 strongly enhanced TL1A mRNA expression but not the expression of CD40, CD70, Ox40L, 4-1BBL, CD80, CD86, B7H1, and ICOSL mRNA (Fig. 4C). Western blotting analysis showed that SP and HK-1 enhance

![Image of a graph showing the expression of TL1A mRNA](http://www.jimmunol.org/)

**FIGURE 3.** SP and HK-1 promote Th17 generation through monocyte activation. IL-17A was quantified in the 7-d supernatants of anti-CD3 mAb-stimulated memory CD4+ T cells, cultured in the presence of monocytes (a; n = 8), with an anti-CD28 mAb (b; n = 5), with monocytes cultured with CD28 mAb-stimulated memory CD4+ T cells in separate chambers (c; n = 4), with 2-d supernatants of SP-, HK-1–, or PGN-activated monocytes, supplemented with an anti-CD28 mAb (d; n = 5), or with monocytes stimulated for 2 d with SP, HK-1, or PGN prior PFA fixation, without (e; n = 4) or with (f; n = 6) the corresponding monocyte supernatants. Results are expressed as the percentage of increase of IL-17A production (mean ± SEM).

**FIGURE 4.** SP- and HK-1–stimulated monocytes potentiate Th17 cell generation through IL-1β, IL-23, and TL1A. A, IL-1β, IL-23, and TNF-α were quantified in the 2-d supernatants of monocytes cultured with 10 μM SP, 10 μM HK-1, or 5 μg/ml PGN. Results are expressed in nanograms per milliliter (mean ± SEM, n = 6). *p < 0.05. B, Memory CD4+ T cells and monocytes were cultured with anti–IL-1β, –IL-6R, –IL-23, –TNF-α, -TL1A, or isotype-matched mAbs. Results are expressed as percentages of inhibition of tachykinin-induced IL-17A production (mean ± SEM, n = 6). *p < 0.05. C, Monocytes were cultured with GM-CSF for 6 h, without or with 10 μM SP or 10 μM HK-1. Expression of the mRNA encoding TL1A, CD40, CD70, Ox40L, 4-1BBL, CD80, CD86, B7H1, and ICOSL was analyzed by qPCR. Results are expressed as the fold increase of mRNA expression in SP- or HK-1–stimulated monocytes compared with nonstimulated monocytes (dotted line) (mean ± SEM, n = 6). D, Monocytes were cultured for 2 d with GM-CSF, without or with 10 μM SP and 10 μM HK-1. TL1A and β-actin production were analyzed by Western blotting. Results are representative of one of four experiments.
TL1A protein expression by monocytes (Fig. 4D). Finally, the potential involvement of TL1A on SP- and HK-1–induced IL-17A production was evaluated in neutralization experiments. Memory CD4+ T cells were stimulated, as described above, in the absence or presence of a neutralizing anti-TL1A mAb. Results showed that the anti-TL1A mAb inhibited SP- and HK-1–induced IL-17A production (inhibition of 79 ± 12 and 91 ± 6%, respectively; mean ± SEM; n = 6) (Fig. 4B, lower panel). Collectively, these results demonstrate that the expression of IL-1β and TL1A induced by SP- and HK-1–stimulated monocytes is critical in the SP- and HK-1–induced human Th17 cell generation.

Discussion
Some neuromediators have emerged as modulators of immune and inflammatory responses. Moreover, the expression of SP, HK-1, and NK-1R is elevated in chronically inflamed tissues. These results suggest that SP and HK-1 may be involved in the initiation and/or regulation of inflammatory reactions. In this study, we report that SP and HK-1 induce the differentiation of human memory CD4+ T cells into Th17 cells through the induction of IL-1β, IL-23, and TL1A expression by monocytes.

Th17 cells, including the bona fide Th17 subset and the Th1/Th17 subset, are characterized by the production of the pro-inflammatory cytokine IL-17. IL-17 induces the production of pro-inflammatory molecules, matrix metalloproteases, and chemokines by numerous cell types. Th17 cells play a critical role in the pathogenesis of many inflammatory disorders, such as psoriasis, RA, multiple sclerosis, and IBD (34). Consequently, to identify the factors involved in Th17 cell generation, it is crucial to design future strategies to treat chronic inflammatory diseases. We showed in this study that the tachykinins SP and HK-1 selectively polarize human memory CD4+ T cells into Th17 and Th1/Th17 cells. Supporting previous studies reporting that SP enhances in vivo IFN-γ production by murine T cells (10, 17), we observed that SP and HK-1 increase IFNγ production by human memory CD4+ T cells. This effect is associated to an increase in the frequency of Th1/Th17 cells, whereas the frequency of Th1 (IFNγ− IL-17A+) cells remains unaffected, thereby showing that SP and HK-1 favor selectively the generation of Th17 and Th1/Th17 subsets.

SP and HK-1 trigger IL-1β, IL-6, and TNF-α production by monocytes. A previous study reported that SP induces the release of these proinflammatory cytokines by human monocytes (11). However, the effect of HK-1 on monocytes remained underdetermined. Our results show that the ability of SP and HK-1 to induce Th17 cells is dependent, at least in part, on the induction of IL-1β production by monocytes. Previous studies have underlined the pivotal role of IL-1β in the differentiation of human memory CD4+ T cells into Th17 cells (29, 37). Nevertheless, in contrast to PGN, we observed that the SP- and HK-1–induced Th17 cell generation is also dependent on monocyte-T cell contacts. The observation that SP and HK-1 induce 20- to 100-fold lower levels of IL-1β than PGN may help explain why additional signals are involved in SP- and HK-1–induced Th17 polarization. We therefore investigated the cell surface molecules involved in the SP- and HK-1–induced Th17 cell generation. SP and HK-1 increase the expression of TL1A on human monocytes, and the role played by TL1A in SP- and HK-1–induced Th17 cell generation was evidenced in neutralization experiments. TL1A is a recently described member of the TNF superfamily. TL1A interacts with DR3, which expression by T lymphocytes and NK cells is upregulated upon T cell activation (38). The TL1A–DR3 interaction has been involved in the pathogenesis of RA and IBD and in a murine model of multiple sclerosis (39–42). Supporting our observations, TL1A–DR3 interaction promotes murine Th17 cell generation (42) and also enhances IFN-γ secretion by human and murine activated CD4+ T cells (43, 44). Finally, we also observed that the production of IL-17A by activated CD4+ T cells cultured with monocytes in the absence of SP and HK-1 is not affected by a neutralizing anti-TL1A mAb. This result indicates that the TL1A–DR3 interaction is involved in the SP- and HK-1–induced Th17 cell generation.

In parallel, we analyzed whether SP and HK-1 modulated the expression of other costimulatory or adhesion molecules on monocytes. SP and HK-1 do not modulate the expression of costimulatory molecules of the TNFR superfamily (CD40), TNF superfamily (4-1BBL, OX40L, and CD70), or B7 family (CD80, CD86, and B7H4) and of the adhesion molecules CD54 and CD62L. SP and HK-1 also failed to modulate the expression of the respective ligands, as well as DR3, on T cells (data not shown).

SP and HK-1 also upregulate IL-23 production by monocytes. IL-23 stabilizes the Th17 phenotype and is required for the maintenance and survival of Th17 cells (26). Accordingly, results indicate that IL-23 is required in SP- and HK-1–induced Th17 cell generation and may contribute to a strong and sustained Th17 response.

In our study, micromolar concentrations of SP and HK-1 were required to induce in vitro the generation of Th17 cells. Several studies have shown that high concentrations of tachykinins are required to activate immune cells, compared with neural cells. NK-1R mRNA is expressed at a low level by human monocytes (46). Moreover, peripheral blood myeloid cells express predominantly a truncated NK-1R variant that exhibits a 10-fold less binding affinity to SP than the full-length NK1R and couples to different and slower intracellular signaling pathways (47, 48). Accordingly, micromolar concentrations of SP are usually used in vitro studies to identify their biological activities on immune cells and especially monocytes (11).

However, several lines of evidence suggest that the concentration of tachykinins can be dramatically increased locally. The amount of SP is increased in sensory nerves supplying localized sites of chronic inflammation (49), and it has been estimated that concentrations as high as 10−7 M may be found locally at nerve endings in the intestine (50). Moreover, SP, which is constitutively expressed by monocytes/macrophages, can be also released by immune cells in the immunological synapse, contributing to generate locally high concentrations of tachykinins required for optimal activation of immune cells. Interestingly, the expression of NK-1R is increased on infiltrating myeloid cells (46), suggesting they are more sensitive to tachykinins than peripheral blood cells. Finally, we can also hypothesize that SP may synergize with pro-Th17 molecules, such as IL-1β, produced locally in inflamed tissues, to contribute to the generation of Th17 cells.

Collectively, these data suggest that, at inflammatory sites, the concentrations of tachykinins are increased and that infiltrating myeloid cells could be more sensitive to tachykinins than peripheral blood cells.

Several studies have reported that the levels of SP are elevated in chronic inflammatory diseases characterized by a massive Th17 cell infiltrate such as psoriasis, RA, or IBD (9, 24, 51). An upregulated expression of NK-1R by RA synoviocytes and in the colon of patients with IBD correlates with the clinical score (9, 24, 25). In a murine model of colitis, SP and HK-1 are also expressed at inflammatory sites (10). We demonstrated in this study that SP and HK-1 act on myeloid cells to induce memory T cells polarization into Th17 cells. As memory T cells and myeloid cells rapidly accumulate at sites of inflammation, it is tempting to
speculate that SP and HK-1 may stimulate locally myeloid cells, thereby favoring the differentiation of infiltrating memory T cells into Th17 cells. The central role of monocytes in the initiation/regulation of Th17 cells has been reported in several studies (27, 28, 52). As an example, memory CD4+ T cells stimulated by an anti-CD3 mAb, in the presence of monocytes or anti-CD28 mAb, give rise to IL-17A production and Th17 cell generation in a cell contact-dependent manner (28). Monocytes isolated from the inflamed joints of patients with active RA spontaneously promote Th17 responses in a cell contact-dependent manner (52). Moreover, monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines, such as IL-1β, that drive IL-17 secretion by memory CD4+ T cells (53). Collectively, these observations suggest that tachykinins, released by the peripheral ending of sensitive nerves and/or by immune cells, may enhance infiltrating myeloid cell activation, proinflammatory cytokine production, and memory Th17 cell generation, contributing to the initiation/maintenance of the inflammatory response. Finally, reinforcing our observations, it has been observed that the treatment with a NK-1R antagonist reduces clinical and histological signs in a murine model of multiple sclerosis (54) and limits the inflammation in a murine model of colitis (55, 56).

In conclusion, this study demonstrates that the tachykinins SP and HK-1 promote the differentiation of memory CD4+ T cells into Th17 cells and thereby suggests that SP and HK-1 could be critical targets in inflammatory disorders to control both proinflammatory cytokine production and Th17 cell generation.

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Disclosures
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References

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