



# Direct action of platelet activating factor (PAF) induces eosinophil accumulation and enhances expression of PAF receptors in conjunctivitis

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**Purpose:** The goal of the present study was to investigate the role of platelet activating factor (PAF) and PAF receptor (PAF-R) in the recruitment of eosinophils into the conjunctiva in the course of PAF induced conjunctivitis. Eosinophils are important players in the immediate hypersensitivity reactions and in allergic conjunctivitis. PAF-R is expressed in many ocular tissues including conjunctival cells. Although it is known that PAF is one of the most potent chemotactic agents for the recruitment of eosinophils, factors responsible for it in conjunctivitis are not clear. Colocalization analysis has been employed to quantify the degree of colocalization of major basic protein (MBP) and PAF-R antigens in the course of PAF induced conjunctivitis.

**Methods:** A 1% solution of PAF was applied in eye drops to male Brown Norway rats. Eyes were harvested with intact conjunctivas at different time points and examined using histology, immunohistochemistry, confocal immunofluorescence microscopy, and reverse transcription-polymerase chain reaction. PAF-R and MBP (a marker of eosinophils) antibodies have been used for immunohistochemical studies. Quantitative analysis of the colocalization of PAF-R and major basic protein (MBP) antigens was performed.

**Results:** Instillation of PAF caused a time dependent recruitment of eosinophils. Eosinophils revealed PAF-R in the intact state. An influx of eosinophils into the conjunctiva was caused by the interaction of PAF with PAF-R and, possibly, with MBP antigen. PAF appeared to enhance the expression of PAF-R by eosinophils and to act toward the PAF-R directly, without chemokine mediation.

**Conclusions:** Quantitative colocalization analysis helped to determine that the recruitment of eosinophils in PAF induced conjunctivitis is accomplished via direct action of PAF toward the PAF-R. It also ensured an objective evaluation of the changes of the degree of colocalization of MBP and PAF-R antigens and the degree of PAF-R expression in dynamics, the findings not otherwise obtainable using qualitative approaches alone.

Upon stimulation, various types of cells, including a majority of cells involved in inflammation and endothelial cells synthesize platelet activating factor (PAF) [1]. PAF has a spectrum of biological activities, many of which are related to the acute allergic and inflammatory response [2]. It also induces synthesis of arachidonic acid and its metabolites [3] and triggers production and effects of various cytokines and eicosanoids [4,5]. Moreover, PAF affects cell adhesion and activation and induces vascular permeability far more potently than histamine [3,4,6]. Importantly, PAF has been identified as the most efficient chemotactic agent for eosinophils [3,7-9].

Compelling evidence has accumulated to date in support of the view that eosinophils are important players in the immediate hypersensitivity reactions and in allergic conjunctivitis [10]. Studies suggested that PAF can directly stimulate rat eosinophils both *in vitro* and *in vivo* [11]. Aerosol and systemic administration of PAF in a number of experimental models

results in extravascular infiltration of eosinophils into the lung which resembles, both in amplitude and duration, the infiltration seen in response to allergen in sensitized animals [12]. Therefore, since allergen exposure of the conjunctiva is followed by the release of PAF, the latter may be related to the allergen induced hyperresponsiveness [10].

The action of PAF is accomplished via a specific receptor coupled with G-protein, which activates a phosphatidylinositol specific phospholipase C [13,14]. It was shown that vascular permeability in the eye and/or cellular infiltration can be reduced by PAF receptor (PAF-R) antagonists [3]. It was also reported that PAF-R mRNA is expressed in the rat corneal epithelium, iris and ciliary body, ganglion cells, inner nuclear layers of the retina, microglia, and choroids [15]. However, in spite of the acknowledged importance of PAF and eosinophils in ocular inflammation, no study to date examined the expression of PAF-R on eosinophils in conjunctivitis.

In the present study, we employed quantitative colocalization analysis of confocal fluorescence microscopy images to evaluate the dynamics of the degree of PAF-R expression by eosinophils and clarify its role in the recruitment of eosinophils. Colocalization of antigens was estimated using three coefficients revealing different aspects of

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colocalization. The following coefficients were used: Pearson's correlation coefficient [16], overlap coefficient according to Manders et al. [16], and overlap coefficients K1 and K2 [17]. We also investigated whether several relevant [18] chemokines, such as IL1, RANTES, eotaxin, MIP 1a, MIP 2, and IL5 are involved into this process.

## METHODS

**Experimental animals:** Male 8-10 week old Brown Norway (BN) rats (Clea, Tokyo, Japan) were used. Animals were kept in a pathogen free colony with water and standard rodent chow available ad libitum. All procedures were executed in adherence to the guidelines of the Research Animal Care Committee of the Kochi Medical School and to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

**PAF administration:** PAF C-18 (1-O-Octadecyl-2-acetyl-sn-glycero-3-phosphocholine) was purchased either from Cayman (Cayman Chemical, Ann Arbor, MI) or from Alexis Biochemicals (Tokyo, Japan) and prepared according to the manufacturer's recommendations. Briefly, PAF powder was dissolved in distilled water and kept as a stock solution at -20 °C. Just before use, it was dissolved in 0.1 M phosphate buffered saline (PBS, pH 7.4) and applied topically in 10 µl eye drops. Initially, for histological examination, PAF was applied at concentrations of 0.2, 1.0, and 2.0%. A 1.0% concentration was chosen as sufficient enough to elicit an inflammatory response. Eyes were collected 30 min, 2, 6, and 24 h following PAF instillations. In controls, PBS was used instead of PAF solution.

**Histology:** Rats were anesthetized and sacrificed by cervical dislocation. Eyes were enucleated with attached lids and intact conjunctiva, fixed with 10% formalin, and embedded in paraffin. Serial 4-5 µm thick paraffin sections were cut along the vertical meridian through the head of optic nerve and stained with May-Giemsa.

**Immunohistochemistry:** The eyes were frozen in hexane cooled with dry ice and immersed in 3% carboxymethyl cellulose (CMC) gel. Then, they were placed in cooled hexane until the CMC gel froze completely. Blocks were kept at -80 °C until further use. After trimming, the surface of the CMC block was covered with a polyvinylidene chloride film (Asahikasei Kogyo, Tokyo, Japan) precoated with synthetic rubber cement, Cryogluce, type 1 (Sakura Finetek, Tokyo, Japan) [19]. After drying, the prepared film was cut with a rotary cutter (Type S, Olf Co., Osaka, Japan). Sections (4 µm thick) were obtained using a Leica CM 3050 S cryomicrotome (Leica Microsystems, Wetzlar, Germany). Sections on films were mounted on cooled slide glasses using double sided adhesive tape (Nitoms, Tokyo, Japan). They were fixed in cooled ethanol for 7 min and washed with 0.1 M PBS, pH 7.4. Blocking buffer (0.1% NaN<sub>3</sub> and 0.3% H<sub>2</sub>O<sub>2</sub> in distilled water) was used to block endogenous peroxidase activity. Sections were then incubated with anti-PAF-receptor (Cayman Chemical, Ann Arbor, MI) and anti-major basic protein (MBP; Biodesign International, Saco, ME) primary antibodies. Incubation lasted for 45 min. After rinsing with PBS, sections were incubated with appropriate biotinylated secondary antibodies for 45 min

at room temperature (RT). Immunostaining was visualized using ABC (Vector Labs, Burlingame, CA) and DAB (Sigma, St. Louis, MO) kits. Finally, sections were briefly stained with hematoxylin. Immunopositive cells were counted throughout the sections. Results were prepared as Excel files and analyzed using Microsoft Excel software.

**Confocal immunofluorescence microscopy:** Sections (6 µm) were cut and fixed as described above. After fixation, they were exposed to 5% goat serum in PBS containing 0.1% Triton X-100 for 30 min to block nonspecific binding. Sections were incubated with the primary antibodies as described above. Then they were rinsed with PBS and exposed to the corresponding secondary antibodies (conjugated with Alexa 488 and Alexa 594; Molecular Probes, Eugene, OR), diluted 1:400, for 45 min at RT in the dark. In controls, primary antibodies were omitted from the labeling process. After a final washing step, sections were mounted with a Vectashield mounting medium (Vector Labs, Burlingame, CA), coverglassed, and examined using a Zeiss Axiovert 135M (Carl Zeiss, Oberkochen, Germany) microscope attached to a LSM 410 confocal laser scanning system (Carl Zeiss, Jena, Germany). The advantage of confocal microscopy is that it generates thin optical sections and is thus able to eliminate the confounding effects of out of focus fluorescence [20]. Most importantly, confocal fluorescence microscopy allows quantification of the colocalization of antigens. Double fluorescence for green and red channels was imaged using excitation of an argon-krypton-neon laser at wave lengths of 488 and 543 nm. Double stained images were obtained by sequential scanning for each channel to eliminate the crosstalk of chromophors and to ensure the reliable quantitation of colocalization.

**Quantitative colocalization analysis:** Confocal images were transferred to a Macintosh Dual PowerPC G5 (Apple Computer, Cupertino, CA) for analysis. Colocalization of antigens was evaluated quantitatively using CoLocalizer Pro (CoLocalizer Pro Software, Boise, ID). Pearson's correlation coefficient (PCC), Manders overlap coefficient (MOC) [16], and overlap coefficients K1 and K2 were employed to evaluate colocalization. Pearson's correlation coefficient (PCC) is one of the standard measures in pattern recognition. It is used for describing the correlation of the intensity distributions between channels. It takes into consideration only similarity between shapes while ignoring the intensities of signals. Its values range between -1.0 and 1.0, where -1.0 indicates no overlap and 1.0 is a complete colocalization [16]. Manders overlap coefficient is a generally accepted measure of colocalization. It indicates an overlap of the signals and thus represents the true degree of colocalization. Values of the MOC are defined from 0 to 1.0. If an image has an overlap coefficient equal to 0.7, it implies that 70% of both its components overlap with the other part of the image. A value of zero means that there are no any overlapping objects [16]. Overlap coefficients K1 and K2 split the value of colocalization into two separate parameters. K1 and K2 coefficients depend on the sum of the products of the intensities of two channels. Thus, they are sensitive to the differences in the intensity of two signals and should be used accordingly [17]. Using our soft-

ware we created histograms and corrected background. Background correction is needed to remove the internal "haze" (pixels of the selected values) from the image. If left in the image, these pixels may be misinterpreted as colocalized. Background was corrected using the threshold value for all channels to remove background and noise levels completely. Then, scatter grams were created and analyzed. Scatter grams estimated the amount of each detected antigen based on colocalization of PAF-R (red, y-axis) and MBP (green, x-axis). Colocalized

pixels of yellow color were located along the diagonal of the scatter gram. At least three samples from each experiment were analyzed. Data were prepared as Excel and image files. Microsoft Excel software was used to analyze Excel files.

*Reverse transcription-polymerase chain reaction:* RNA was extracted from conjunctivas using a commercially available homogenizer (Mixer Mill MM 300; Qiagen KK, Tokyo, Japan) and then transcribed into cDNA. PCR was performed using a DNA thermal cycler (Applied Biosystems, Foster City,

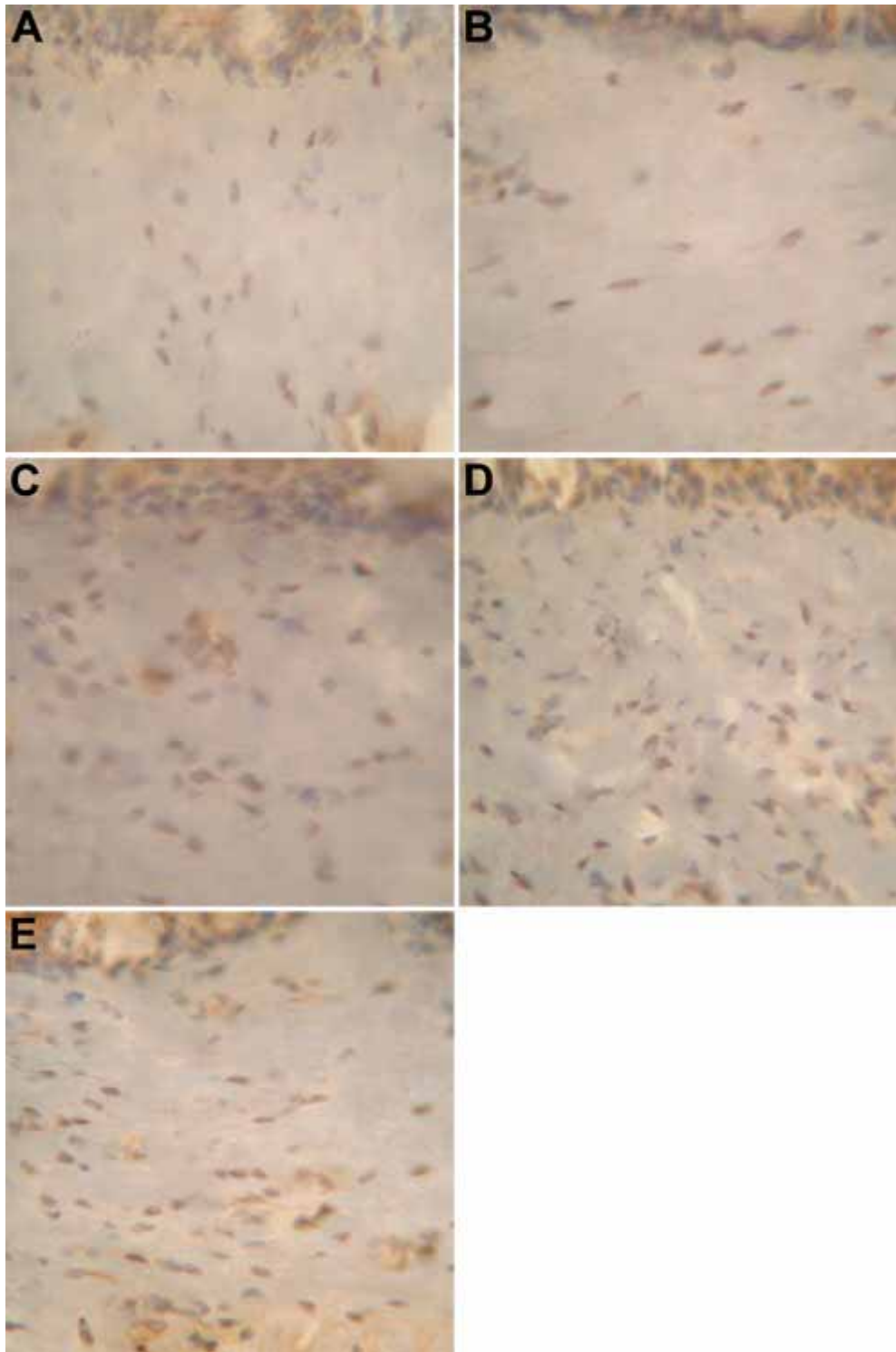


Figure 1. Immunohistochemical visualization of PAF-R antigen. A control is shown in A. An increase of the number of PAF-R positive cells is seen at 30 min (B) and 2 h (C) after PAF administration. The number of the cells increases considerably at 6 h (D). It continues to grow by 24 h (E). Representative images of three examined eyes are shown. Magnification x400.

CA) using a 1 min cycle at 94 °C followed by 30 to 40 cycles consisting of denaturation at 94 °C for 30 s, annealing at the optimal temperature of the primer pairs used for 30 s, and extension at 72 °C for 90 s. The following molecules were examined; IL 1b, IL 1RA, CCL5 (RANTES), CCL10 (eotaxin), MIP 1a, MIP 2, and IL5. The primer pairs [21-26] were either synthesized by Sawady Technology (Tokyo, Japan) or purchased from Biosource International Inc. (Camarillo, CA).

After 40 cycles of amplification for IL 1, RANTES, and eotaxin, MIP 1a, MIP 2, and IL5 PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide. Gels were photographed and examined. RT-PCR for  $\beta$ -actin for 30 cycles was performed as a control. As a molecular marker, X174/*Hae*III digest (Wako, Osaka, Japan) was used.

**Statistical analysis:** Statistical comparisons of the number of infiltrating cells and the degrees of colocalization were performed using the Mann-Whitney U test. A  $p < 0.05$  was considered to be statistically significant.

## RESULTS

**Clinical response of the eyes:** Instillation of 1% PAF caused severe edema of the lids, conjunctival redness, and chemosis. These symptoms started appearing approximately 30 min after PAF administration, reaching a peak at 2 h. By 6 h, the severity of inflammatory reaction began decreasing. At 24 h, the signs of inflammation were minimal (data not shown).

**Histology:** Histological examination of the conjunctivas clearly showed a marked increase of the number of infiltrating cells as a result of PAF administration. The increase was time and dose dependent (data not shown).

**Immunohistochemistry:** Anti-PAF-R and anti-MBP antibodies were used to visualize cells expressing PAF-R and eosinophils, respectively. Both antibodies clearly recognized their respective antigenic sites, although the staining pattern differed. Staining of MBP antigen appeared to be stronger than that of PAF-R antigen. As compared to controls, administration of PAF yielded a slight increase in the number of PAF-R positive cells at 30 min and 2 h after PAF instillation. At 6 h, however, the number of the cells increased more than three times. By 24 h, the number of PAF-R positive cells continued to grow significantly (Figure 1, Figure 2B). In controls, only a few cells were MBP positive. After PAF administration (30 min), the number of eosinophils more than doubled. Moreover, 2 h after PAF was instilled, the number of the cells increased and kept increasing until 6 h. Samples taken 24 h af-

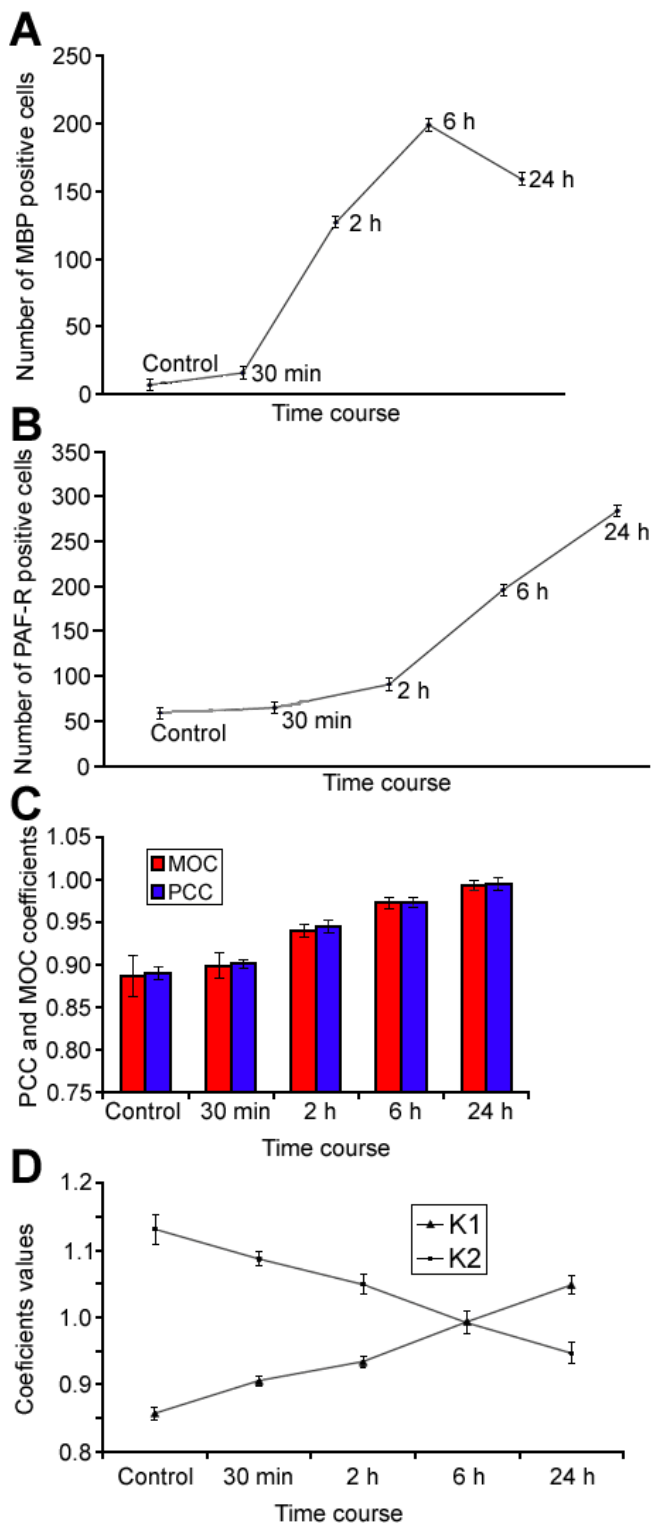


Figure 2. Dynamical changes of the number of PAF-R and MBP positive cells and colocalization parameters. Quantitative representation of the changes of the number of PAF-R and MBP immunopositive cells (A and B), Manders' colocalization and Pearson's correlation coefficients (C), and overlap coefficients K1 and K2 (D) in dynamics of PAF induced conjunctivitis. A and B document an increase of the number of MBP and PAF-R positive cells with their respective peaks at 6 and 24 h after PAF administration. Values at each time point were compared to values at all other time points. All of these comparisons were found to be statistically significant ( $p < 0.01$ , Mann-Whitney U test). C and D reveal changes of colocalization of PAF-R and MBP antigens. Manders' Overlap Coefficients (MOC) and Pearson's correlation coefficients (PCC) indicate a steady increase of the degree of colocalization during the whole period of observation (C), while overlap coefficients K1 and K2 indicate profound changes of the contribution of PAF-R and MBP antigens to their colocalization (D). Values at each time point were compared to values at all other time points. All of these comparisons were found to be statistically significant ( $p < 0.05$ , Mann-Whitney U test). Data are an average of three samples examined for each time point.



ter PAF administration displayed no further increase in the number of eosinophils, on the opposite, their number started to decrease (Figure 3, Figure 2A). Omission of the primary antibodies in controls of specificity of immunohistochemical visualization showed no staining.

*Confocal immunofluorescence microscopy:* Confocal immunofluorescence microscopy of double stained sections at each time point was employed to confirm the results of immunohistochemical staining and to obtain more precise information on the expression of PAF-R and MBP antigenic sites. PAF-R and MBP antigens colocalized at all times studied and

in all examined samples, including controls (Figure 4). Controls of specificity of immunofluorescence detection utilizing secondary antibodies alone resulted in the absence of detectable fluorescence.

*Quantitative colocalization analysis:* As confocal microscopy showed that PAF-R and MBP antigens were colocalized to a various degree in controls and changed the pattern of colocalization in the course of PAF induced conjunctivitis, quantitative colocalization analysis of these images was performed. It revealed a noticeable increase in the degree of colocalization of the PAF-R and MBP antigens in the course

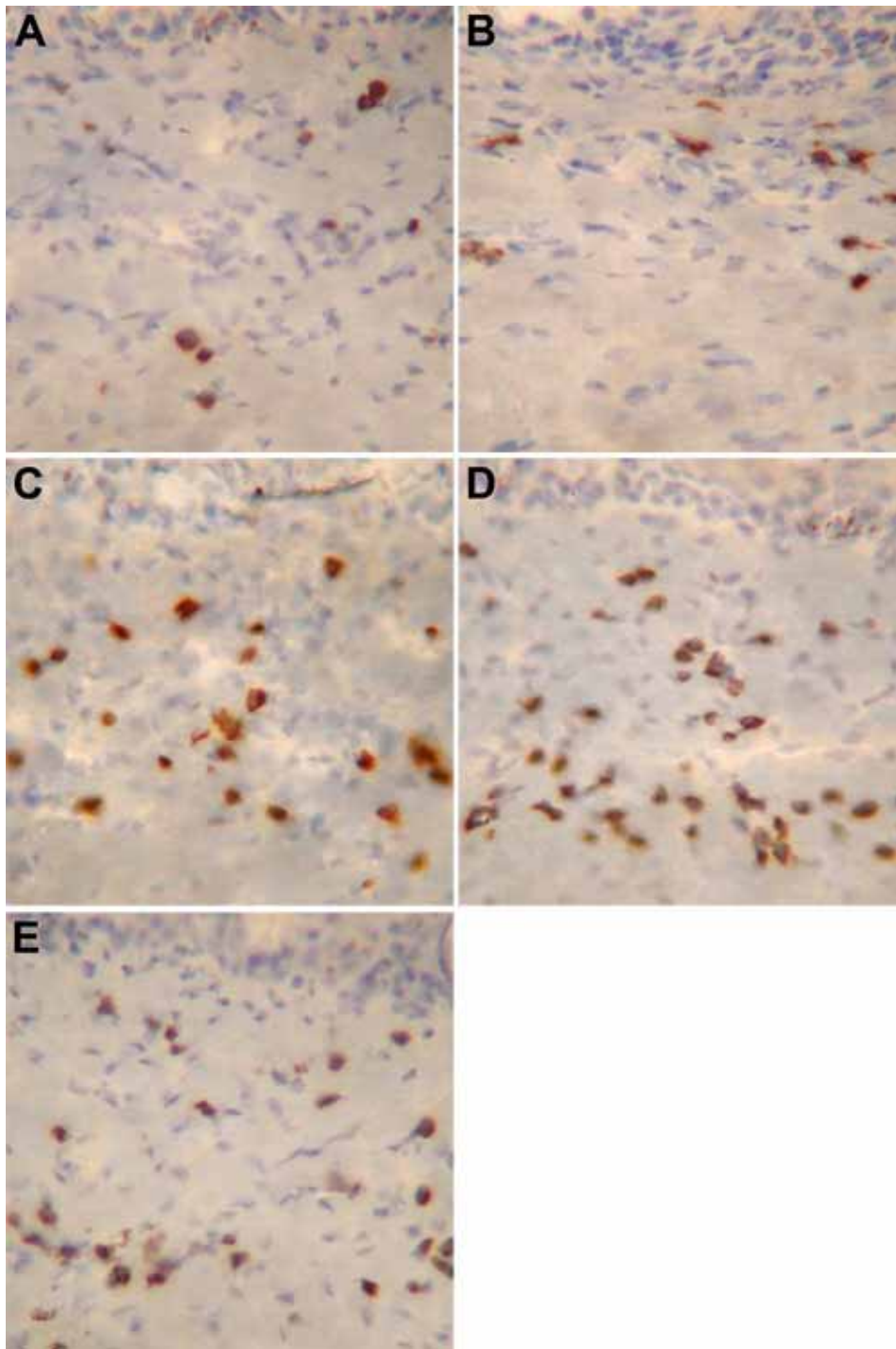


Figure 3. Immunohistochemical visualization of MBP antigen. MBP positive cells are seen occasionally in controls (A). The number of MBP positive cells more than doubles at 30 min after PAF challenge (B). It increases dramatically at 2 h (C) and keeps increasing until 6 h (D). At 24 h, a decrease in the number of immunopositive cells is noticed (E). Representative images of three examined eyes are shown. Magnification x400.

of PAF induced conjunctivitis (Figure 2C). In intact animals, PCC was 0.890, MOC was 0.886. At 30 min after PAF administration, PCC was 0.901, MOC was 0.899, and at 2, 6, and 24 h the coefficients were 0.945, 0.939, 0.973, and 0.972, 0.992, 0.995, respectively. In control animals, coefficient K1, reflecting the impact of PAF-R antigen, was 0.857. After PAF instillation (30 min), K1 was 0.905, at 2, 6, 24 h it was 0.934, 0.994 and 1.048, respectively. K2 coefficient, reflecting the impact of MBP antigen to the process of colocalization, was 1.131 in the intact animals. At 30 min, 2, 6, and 24 h K2 was 1.087, 1.049, 0.992 and 0.947, respectively (Figure 2D). The scatter

gram in the upper right corner of each image (Figure 4) estimated the amount of each detected antigen based on colocalization of PAF-R (red, y-axis) and MBP (green, x-axis). Colocalized pixels of yellow color were located along the diagonal of the scatter gram and showed significant colocalization of antigens even in controls.

*Reverse transcription-polymerase chain reaction:* To address the mechanism of eosinophil recruitment into the conjunctiva, we examined the expression of mRNA of IL1, RANTES, eotaxin, MIP 1a, MIP 2, and IL5 in control samples and 30 min, 2, 6, and 24 h after PAF administration. mRNA

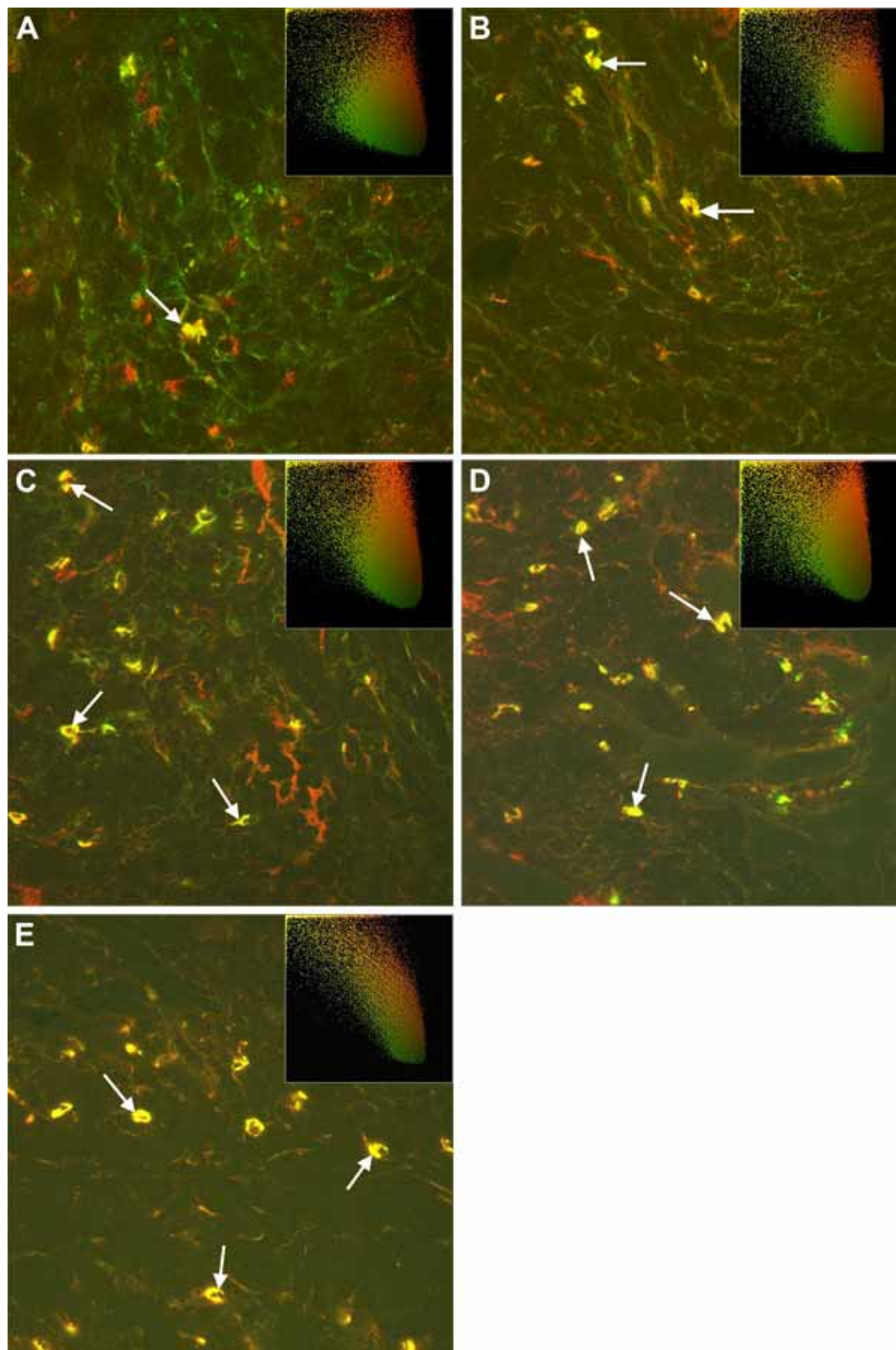


Figure 4. Double staining for PAF-R and MBP antigens. Confocal immunofluorescence microscopy of conjunctiva sections stained with anti-PAF-R (red fluorescence) and anti-MBP (green fluorescence) antibodies. The embedded scatter gram in the upper right corner of each image estimates the amount of each detected antigen based on colocalization of PAF-R (red, y-axis) and MBP (green, x-axis). Colocalized pixels of yellow color are located along the diagonal of the scatter gram. All images reveal colocalization of PAF-R and MBP antigens at different time points (arrows, A-E). Magnification x400.

expression in all samples remained approximately constant in all examined samples and at all time points, showing signs of neither upregulation nor downregulation. This result suggests that IL1, RANTES, eotaxin, MIP 1a, MIP 2, and IL5 are not involved in the process of eosinophils recruitment into the conjunctiva following PAF challenge (Figure 5).

### DISCUSSION

Analysis of colocalization of antigens has become one of the most widely noticed and highly credited observations in modern cellular and molecular biology. Although colocalization is relative, information on the appearance of distinct molecules at the same location can be of particular significance, thus helping to draw important conclusions about their interactions and functional compatibility. Usually these conclusions are

based merely on naked eye evaluation, without any quantitative justification. Lately, several studies have appeared which attempted to evaluate colocalization quantitatively [27-30]. However, these studies evaluated only a single colocalization coefficient and provided a narrow, static look at the functional significance of colocalization.

We examined the role of PAF, PAF-R, and the relevant chemokines in eosinophil recruitment into the conjunctiva in the course of PAF induced conjunctivitis and used the developed software to evaluate the colocalization of PAF-R and MBP antigens. It was found that topical administration of PAF causes infiltration of eosinophils and potentiates the expression of PAF-R by them in a time dependent manner. Recently, we used quantitative colocalization analysis to explore the dynamics of expression of PAF receptor in PAF induced con-

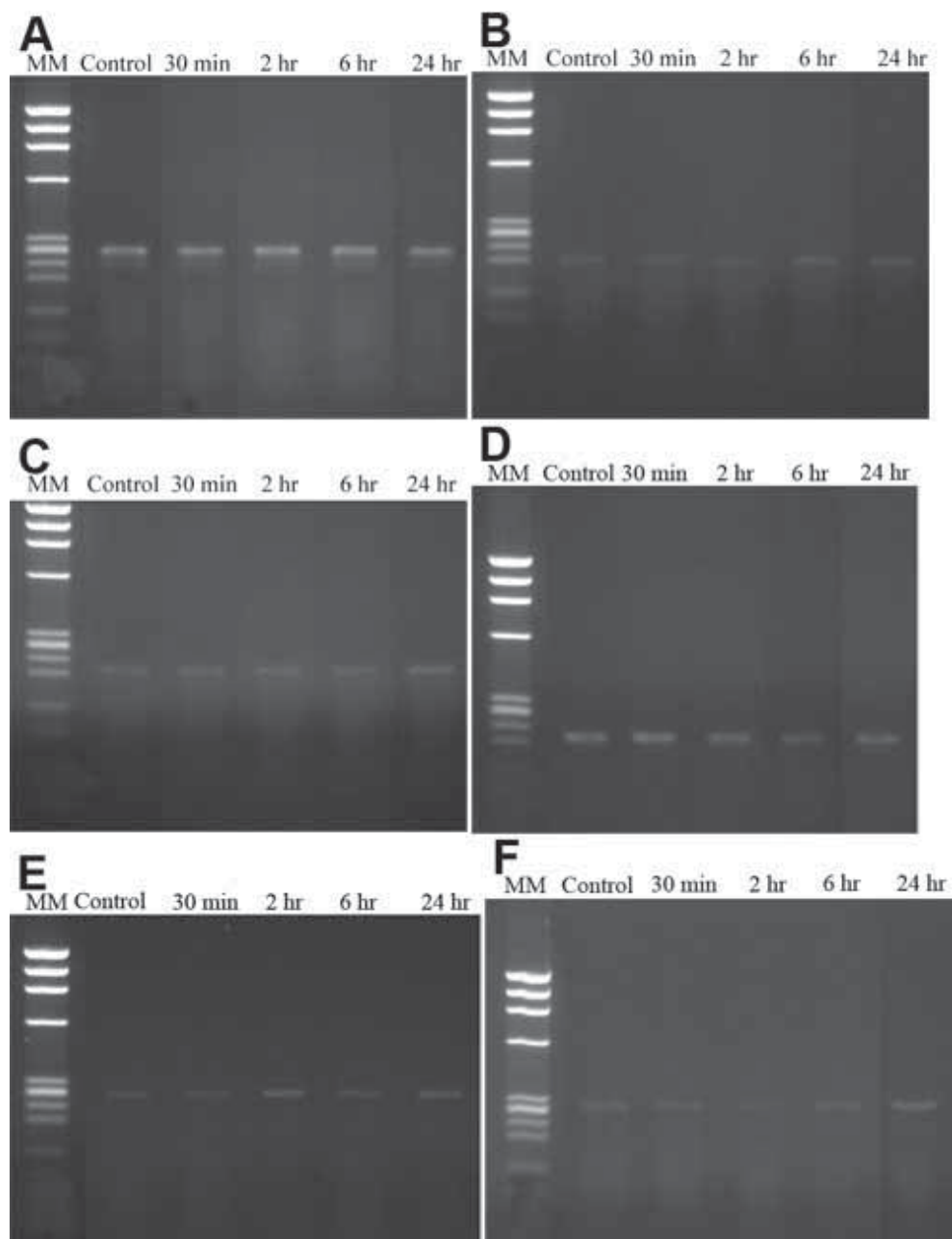


Figure 5. Expression of IL1, RANTES, eotaxin, MIP 1a, MIP 2, and IL5 in the conjunctiva following PAF challenge. Conjunctiva were harvested 30 min, 2, 6, and 24 h after PAF administration and subjected to RT-PCR analysis. Synthesis of IL1 (A), RANTES (B), eotaxin (C), MIP 1a (D), MIP 2 (E), and IL5 (F) mRNA is not affected at all examined time points. Representative data of three examined samples are shown.  $\beta$ -Actin was used as a control.



conjunctivitis [31]. The present study was focused on eosinophils because these cells are believed to be the key element in immediate hypersensitivity reactions and in asthma [10]. Eosinophils are also the predominant cell type in human tear fluids during the late phase inflammatory responses to antigen in allergic subjects [32]. At the same time, PAF is described as the most potent endogenous chemotactic agent for eosinophils [7]. A number of studies have shown that PAF, a phospholipid cytokine, is able to generate inter- and intracellular signals. These signals mediate various cell functions acting via a specific receptor coupled with G-protein [13,14]. Honda et al. [13] cloned and expressed a guinea pig lung PAF-R and reported that it belongs to the G-protein coupled receptor family. Penido et al. [33] showed that LPS induced eosinophils accumulation depends on G-protein coupled receptor activation through the mechanism independent on eotaxin, RANTES, or other chemokines. Other studies reported that blocking PAF-R is an efficient way to control the inflammatory response in various ocular tissues [3,7]. However, the factors responsible for PAF induced infiltration of eosinophils, such as the role of chemokines and interaction of PAF and PAF-R, were not evaluated. Furthermore, quantitative colocalization analysis has never been used to clarify the changes of the degree of colocalization of PAF-R and MBP antigens and the degree of expression of PAF-R in dynamics.

Confocal immunofluorescence microscopy showed the expression of PAF-R on eosinophils in all examined samples and at all time points, including controls, indicating the presence of PAF-R on eosinophils even in the intact state. Quantitative colocalization analysis demonstrated a high degree of colocalization even in controls. Eventually, it showed a gradual increase as the PAF induced inflammation developed. Importantly, the two coefficients, while revealing the different aspects of the colocalization process, showed a similar pattern of changes. Interestingly, when the number of infiltrating eosinophils started to decrease and clinical signs of the inflammatory reaction almost vanished (by 24 h after PAF administration), the degree of colocalization still increased. Does this indicate that eosinophils found in the conjunctiva sections at this time point maintain an elevated expression of PAF-R? Or does this show the changes of the expression of MBP antigen? To answer these questions we calculated another coefficients, K1 and K2. It was found that the degree of PAF-R expression was gradually increasing during our experiment, while expression of MBP antigen decreased. These data gave reason to conclude that administration of PAF caused an increasing degree of PAF-R expression by eosinophils even when the severity of the inflammatory reaction is fading. Interestingly, the peak of the number of infiltrating eosinophils detected using histological examination coincided with the moment, at 6 h after PAF administration, when K1 equaled K2, meaning the intensity of MBP and PAF-R antigens' signals was the same. After this period of time, K1 continued to grow, but the number of infiltrating eosinophils was not increasing any longer. This may imply that the interaction of both PAF-R and MBP antigens with PAF is required for the recruitment of eosinophils.

Since exposure to antigens in the eye releases mediators promoting the influx of eosinophils into the conjunctiva, the interaction of them, in particular IL1, RANTES, eotaxin, MIP 1a, MIP 2, and IL5, and PAF with these cells may be related to eosinophil infiltration. Therefore, we examined the expression of IL1, RANTES, eotaxin, MIP 1a, MIP 2, and IL5 mRNA at the selected time points. The examined cytokines play an important role in the recruitment of eosinophils to the sites of inflammation and are related to the activation of these cells [11,18,33-40]. We observed that PAF administration had no effect on IL 1 mRNA expression. This finding was rather unexpected, because IL 1 is known as one of the key inflammatory cytokines playing an important role in the immune response. IL 1-like peptide is synthesized by ocular cells and was detected in the fluids from the sites of ocular inflammation and injury [34]. It was reported that IL 1 may play an important role in the recruitment of eosinophils to the sites of allergic inflammation [11]. Our observations are corroborated by the data from other laboratories that PAF itself is unable to evoke production of IL 1 [1] and agree with studies reporting that PAF may act directly as a chemotactic agent for eosinophils [1,7].

Other mediators, RANTES and eotaxin, are closely related not only to chemotaxis but also to the activation of eosinophils manifested by degranulation, an increase of oxidative metabolism, and adherence [35-37]. Results of our RT-PCR experiments did not reveal any upregulation of the synthesis of these two chemokines' mRNA, suggesting that infiltration of eosinophils was influenced neither by RANTES nor by eotaxin.

Macrophage inflammatory protein (MIP)-1a is a known chemotactic agent for eosinophils, monocytes, and lymphocytes [18,33]. Eosinophils are also targets for another chemokine MIP2 [18]. No upregulation of the synthesis of the mRNA for these chemokines has been revealed by RT-PCR experiments. We have not found any upregulation of the mRNA synthesis of another chemokine, IL5, known as a potent chemotactic factor for eosinophils [38-40]. Thus, the direct action of PAF toward PAF-R is a strong feasibility and is supported by the findings of Penido et al. [33] that eosinophil accumulation depends on G-protein coupled receptor activation through a mechanism independent of eotaxin, RANTES or other chemokines. This agrees with a number of studies reporting that PAF can directly stimulate rat eosinophils in vitro and potentiate the generation of eosinophil chemotactic activity in the rat model [1,7,11]. It is worth mentioning that PAF and allergen induced eosinophils infiltration in the guinea pig appears to be platelet dependent, because it can be significantly reduced by pretreatment of animals with cytotoxic anti-platelet antibody or anti-platelet drugs [7]. A number of experimental studies suggested that various antagonists of PAF-R can inhibit inflammatory reactions [4,7,12]. Treatment with SRI 63-441, a PAF-R antagonist, inhibited interleukin 1 induced increase in vascular permeability and leukocyte infiltration in the rabbit eye following intravitreal injection of human interleukin 1-alpha [9]. It was reported that this antagonist can reduce ocular inflammation without inhibiting pros-



taglandin E2 synthesis and can be combined with a topical corticosteroid or a topical cyclooxygenase inhibitor to produce an additional reduction of inflammation [3,9]. A topical administration of another PAF-R antagonist, BN 52021, limits inflammation caused by laser trauma of the iris [41]. Therefore, pharmacologic agents that inhibit PAF synthesis or its binding to PAF-R may prove to be useful for the control of ocular inflammation. We did not intend in the present study to elucidate the applicability of PAF-R antagonists, however, the quantitative colocalization analysis reported here should serve as a useful tool for clarification of the mechanisms of their action in other investigations. Finally, the fact that we are able to quantify the degree of colocalization of MBP and PAF-R antigens and the degree of PAF-R expression changes in dynamics, allows determination of the proper time frame for the use of PAF-R antagonists in clinical settings.

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