Volume 257, number 1, 49–51 FEB 07715 October 1989

Presence of paf-acether in human thymus

Patrick Salem, Yves Denizot, Christina Pitton, Anne Dulioust, Marie-Jeanne Bossant+, Jacques Benveniste and Yolene Thomas

Institut National de la Santé et de la Recherche Médicale, U 200 Université Paris-Sud, 32 rue des Carnets, 92140 Clamart, France

Received 23 July 1989

Paf-acether (paf) is a phospholipid mediator of inflammation endowed with major immunoregulatory properties. The present study demonstrates that human thymus contains large amounts of paf, as well as paf precursors. In addition, isolated thymic cells produced paf under ionophore stimulation. Paf from thymus exhibited the same biological and physicochemical properties as synthetic paf. The purity and molecular structure of paf from thymus were further characterized by reverse-phase HPLC and gas chromatography with electron-capture detection. These findings may have important implications since thymus microenvironment is essential in the proper development of bone marrow progenitors committed to the T cell lineage into thymocytes capable of emigrating to the periphery as functional T lymphocytes.

Platelet-activating factor-acether; Thymus

1. INTRODUCTION

Paf-acether (paf, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a phospholipid mediator of inflammation that is released from and activates a variety of inflammatory cells such as platelets, neutrophils, eosinophils and monocytes/macrophages [1–3]. Recently, several effects of paf on the immune response have been unveiled. For example, paf modulates cell surface structures that play a crucial role in T cell function and interferes with the processes leading to T cell activation [4,5]. It was thus of interest to investigate whether paf could be synthesized in the whole thymus. Indeed, thymus is an anatomically complex organ critical for the development of immune function in young mammals. It is composed of an epithelial stroma, thymocytes, dendritic cells, macrophages and connective tissue. Although a great deal is known about the cell types that occupy the thymus and their interactions, the signals involved in the processes of lymphocyte maturation are still to be elucidated [6].

In the present study, we demonstrate that human thymus contains large amount of paf and that isolated thymus cells produce paf under ionophore stimulation. These results could be of importance for the understanding of human intrathymic T cell differentiation.

Correspondence address: Y. Thomas, INSERM U 200, 32 rue des Carnets, 92140 Clamart, France

+ *Present address:* National Institute on Alcohol Abuse, and Alcoholism, DICBR; Laboratory of Clinical Studies, Bethesda, MD 20892, USA

This paper is fifth of a series: Immunoregulatory functions of paf-acether

2. MATERIALS AND METHODS

2.1. *Processing of thymus tissue*

Thymus were obtained from 3-year-old patients who had part of their thymus removed during cardiac surgery. Portions of the thymus were weighed, minced into small pieces and then washed in saline to remove blood cells. Subsequently, the minced thymus tissue was pulverized and homogenized in methanol at 4°C. Lipids were then extracted using the method of Bligh and Dyer [7]. Phospholipids were purified by normal-phase high performance liquid chromatography (HPLC) and different fractions assayed for platelet-aggregating activity [8].

2.2. *Isolation of thymic cells*

Single cell suspensions were obtained by mincing a portion of the thymus and then pressing the fragments through a stainless steel mesh as described previously [9]. This population was shown to contain more than 95% $CD2^+$ cells. Isolated thymic cells, 1 to 50×10^6 cells/ml, were stimulated by 2 μM ionophore A23187 (IoA) (Calbiochem, La Jolla, CA) at 37°C in Hepes-buffer saline (HBS) containing 2.5 mg/ml fatty acid-free bovine serum albumin (Sigma Chemical Co., St. Louis, MO) [10]. After various periods of time, total paf was recovered using ethanolic extraction of both supernatants and cell pellets, as described [10].

2.3. *Paf assay*

Washed rabbit platelets were prepared as in [11]. Aspirinated-platelets (1.6×10^8) in Tyrode's buffer (300 μl) containing 2.5% gelatin and the ADP scavenger mixture, creatine phosphate (1 mM)/creatine phosphokinase (10 U/ml) were stirred in an aggregometer (Icare, Marseille, France). Aggregating activity of the samples was measured over the linear portion of the calibration curve obtained with 5–50 pg synthetic paf (Bachem, Bubendorf, Switzerland).

2.4. *Assay for lyso paf and 1-alkyl-2-acyl-glycerophosphocholine (AAGPC)*

Lyso paf was measured in samples after its chemical acetylation in-

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00145793/89/$3.50

to paf as previously described [10]. The amount of lyso paf was established as the difference between the quantity of paf measured before and after acetylation of the samples. AAGPC was measured after alkaline hydrolysis and acetylation as described [10].

2.5. *Gas chromatography with electron capture detection (GC-ECD)*

Samples obtained after HPLC separation were hydrolysed to the diglycerides with phospholipase C from *C. welchii*, followed by heptafluorobutyrate (HFB) derivatization. GC-ECD was performed using a Girdel 30 instrument (Delsi, Suresnes, France) and an OV 1 fused silica capillary column (0.25 mm id × 25 m long) maintained at 215°C. The temperature of the capillary injector was kept at 270°C. The capillary head pressure was set at 0.4 bar with helium as carrier gas. For ECD, nitrogen was used as a make-up gas at a flow rate of 30 ml/min and the 10 mCi ^{63}Ni detector was maintained at 300°C [12].

2.6. *Statistical analysis*

Means of at least 3 independent experiments were compared using the Student's *t*-test for paired variables.

3. RESULTS

Lipids were extracted from thymus and subjected to normal phase HPLC. Paf was quantitated by bioassay as described in section 2. As illustrated in table 1, paf content in thymus ranged from 38.80 to 97.19 pmol/g wet wt (n = 6). The concentration of AAGPC in thymus was several orders of magnitude greater than that of lyso paf and paf. The molecular ratios of paf to lyso paf and to AAGPC were 1:82 and 1:1633, respectively. The bioactive paf material was characterized as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by the following biological and physicochemical criteria: (i) the aggregating activity found in the extract was totally inactivated by treatment with phospholipase A_2 but not with lipase A_1 [13]; (ii) two paf antagonists L-652,731 (generously provided by Dr J. Chabala, Merck Sharp and Dohme, Rahway, NJ) and WEB-2086 (obtained from Boehringer, Ingelheim, FRG) inhibited submaximal platelet aggregation induced by thymus and synthetic paf. Furthermore, the active fractions eluted from normal-phase HPLC were pooled and analysed using GC-ECD. As depicted in fig.1, two peaks were identified and assigned to 16:0 paf (85%) and 18:0 paf (15%). Similar results were obtained for aggregating samples obtained after chemical treatment of AAGPC or lyso paf (not shown).

Additional experiments shown in fig.2 indicate that unfractionated thymic cells were able to produce paf

Table 1

Paf and paf precursors in human thymus

Paf (pmol)	Lyso paf (nmol)	AA-GPC (nmol)
67.2 ± 14.7	5.5 ± 2.2	110.7 ± 12.5

Lipids were extracted from thymus and subjected to normal phase HPLC. Paf and paf precursors were quantitated by bioassay as described in section 2. The results are expressed per g wet wt (means ± SE of tissue samples from six donors)

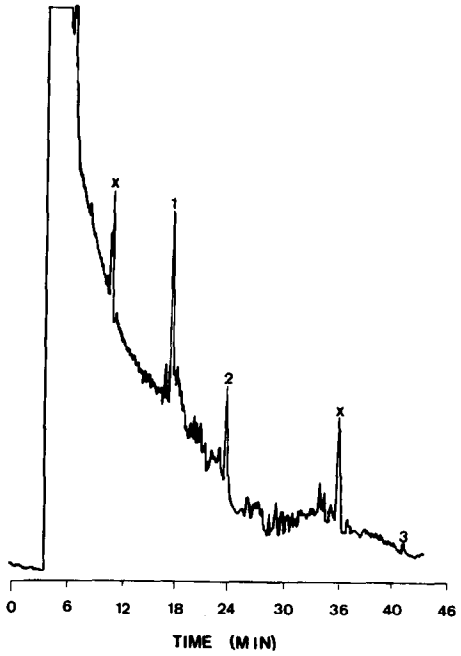

Fig.1. GC-ECD obtained from heptafluorobutyrate derivatives of paf extracted from thymus. 1, O-CH_3 C16 paf (internal standard); 2, 16:O-paf; 3, 18:O-paf; x, unknown compounds.

spontaneously. In the presence of 2 μM IoA, the amount of paf increased sharply within the first 2 min of stimulation, plateaued from 20 to 30 min and decreased thereafter. Of note, paf level at 20 min was null when 4×10^6 cells/ml/tube were used but rose to 2.5 ± 0.73 pmol (mean ± SE, n = 4, $P < 0.01$) for 1×10^7 cells and reached a plateau of 6.12 ± 1.5 pmol for 4 to 10×10^7 cells ($n = 4$, $P < 0.01$). One interpretation of this observation is that only a minor fraction of the whole thymic population actively produces paf. Indeed, when thymic cells were enriched in non-lymphoid

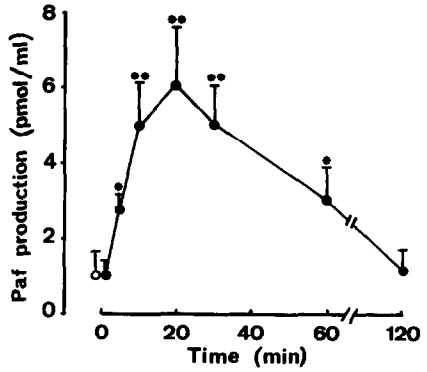

Fig.2. Time course of paf production by IoA-stimulated thymocytes. Isolated thymic cells (40×10^6/ml) were incubated for varying periods of time at 37°C either in the presence (●—●) or in the absence (○) of 2 μM IoA. Total paf (released + cell-associated) was recovered using ethanolic extraction and then measured by aggregation of washed rabbit platelets as described in section 2. The mean ± SE of 7 different experiments is indicated. *P*-values were * $P < 0.01$, ** $P < 0.001$ for stimulated cells as compared to unstimulated cells.

populations (CD2-negative cells), 7.12 ± 1.0 pmol paf was obtained for 1×10^6 cells ($n = 3$).

The material extracted from thymic cells after ionophore stimulation exhibited biological and physicochemical properties identical to those of either synthetic paf or paf extracted from the whole thymus according to the criteria described above. Reverse-phase HPLC and gas chromatography also indicated that most of the paf generated was of the hexadecyl species (not shown).

4. DISCUSSION

The present results indicate that human thymus contains large amounts of paf as well as paf precursors, AAGPC and lyso paf. Identification of paf was based on the stringent functional and biophysical criteria detailed above including reverse-phase HPLC and gas chromatography analysis. It is unlikely that circulating blood accounted for the amount of paf found in the thymus since: (i) small fragments of tissue were extensively rinsed till blanching before being homogenized in methanol; (ii) the amount of bioactive paf found in blood was 16.2 ± 4.3 pmol/g, mean ± SE of samples from 10 individuals; (iii) isolated thymic cells produced paf spontaneously and the amount of paf was highly enhanced by the addition of IoA to the culture. Two specific enzymatic reactions have been documented to be involved in paf biosynthesis. An acetyl-CoA acetyltransferase that catalyzes the acetylation of inactive lyso paf into the bioactive paf whereas a CDP-choline cholinephosphotransferase transfers the phosphobase from CDP-choline to alkyl-acetyl-glycerol [14]. Whether the thymic paf originates from the de novo or the remodeling pathway remains to be investigated. However, it is interesting to note that Wykle et al. have previously reported that rat thymus contained acetyltransferase activity in high amounts [15].

At the present time, the precise cellular origin of thymus paf remains an open question. One possibility is that it originates from nonlymphoid populations such as dendritic cells, macrophages or epithelial cells. An alternative view is that thymocytes themselves can form paf. We favor the former interpretation since our results indicate that CD2-negative cells were far more efficient than CD2-positive cells in producing paf. This does not, however, eliminate the possibility that subsets of thymocytes could also produce paf. Clearly, additional studies using distinct isolated and/or cloned cell populations will be necessary to address these issues.

The potential significance of these findings must be considered in light of the role of thymus in T cell ontogeny along with our recent studies demonstrating that paf modulates T cell function. For example, we showed that paf inhibited T cell activation induced via the CD3 pathway (antigen-restricted) but potentiated that induced via the CD2 pathway (antigen-unrestricted) (Vivier et al., submitted). Thus, it is conceivable that paf may regulate the ability of thymocytes to participate in CD3- and CD2-restricted interactions [16]. Regardless of the exact role of paf in the thymus, these data, when considered together, indicate a more fundamental role for paf than the now classical ones in allergy and inflammation and point out paf as a candidate in the regulation of intrathymic T cell differentiation.

Acknowledgements: We are grateful to Professor P. Neveux and the operating room staff of Laennec's Hospital, Paris, for providing thymus specimens.

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