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Pre-B Cell Colony Enhancing Factor (PBEF), a Cytokine with Multiple Physiological Functions

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Abstract

Pre-B cell colony enhancing factor (PBEF) is regarded as a proinflammatory cytokine. Named for its first discovered function as a pre-B cell colony enhancing factor, it has since been found to have many other functions relating to cell metabolism, inflammation, and immune modulation. It has also been found to have intracellular and extracellular forms, with the two overlapping in function. Most of the intracellular functions of PBEF are due to its role as a nicotinamide phosphoribosyltransferase (Nampt). It has been found in human endothelial cells, where it is able to induce angiogenesis through upregulation of VEGF and VEGFR and secretion of MCP-1. In human umbilical endothelial cells, PBEF increases levels of the protease MMP 2/9. PBEF has also been found in a variety of immune cells other than B cells and has been shown to inhibit apoptosis of macrophages. Extracellular PBEF has been shown to increase inflammatory cytokines, such as TNF- α , IL-1 β , IL-16, and TGF- β 1, and the chemokine receptor CCR3. PBEF also increases the production of IL-6, TNF- α , and IL-1 β in CD14⁺ monocytes, macrophages, and dendritic cells, enhances the effectiveness of T cells, and is vital to the development of both B and T lymphocytes. The purpose of this review is to summarize the recent advances in PBEF research.

Keywords

pre-B cell enhancing factor; cytokine; chemokine; monocyte; B lymphocyte; T lymphocyte

Introduction

Since its discovery in 1994, PBEF protein has been found to have several intracellular and extracellular functions (1). It has three central physiological functions relating to cell metabolism (2–3), inflammation (4), and immune modulation (5). In the intracellular compartment, it functions mainly as nicotinamide phosphoribosyltransferase (Nampt) (2, 6), which is the first enzyme and the rate-limiting step in the nicotinamide adenine dinucleotide

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(NAD⁺) salvage pathway from nicotinamide. NAD⁺ is then converted to NADP, which is then converted to NADPH. Through an unknown mechanism, PBEF is released to the extracellular environment (7–8), where it has been shown to increase the production of inflammatory cytokines, such as IL-8 (7), IL-6 (5), IL-1, and TNF-. PBEF's immune-modulating properties can be either intracellular or extracellular. Its role in innate immunity has been characterized extensively, but it also plays a role in adaptive immune responses.

In addition to its physiological functions, PBEF is also involved in a number of pathological states. It has been implicated in several inflammatory diseases, including psoriasis (9), rheumatoid arthritis (10), and ulcerative colitis (5). It has also been shown to play a role in atherosclerosis (11), to be extensively linked to acute lung injury in sepsis patients (12), and is implicated in preterm births (13). It has been hypothesized that PBEF plays a role in type 2 diabetes mellitus (T2DM) (14). However, despite much work in this area, conflicting clinical data and a recalled paper (15) indicate that this association remains controversial and not well understood. PBEF has also been linked to various cancers *via* its Nampt activity (16).

Characteristics of the PBEF gene and protein structure

Upon first discovery of PBEF, much work was carried out to characterize its gene and protein structure in humans (1). Samal determined that there are 3 mRNA variants, with lengths of 2.0, 2.4, and 4.0 kilobases (kb), transcribed by the PBEF gene. The 2.4-kb variant is the most abundant and its open reading frame encodes a protein of 473 amino acids (aa) in length, with a predicted size of ~5.2 kDa, which was confirmed experimentally. The 3' untranslated region is 69% adenine/thymine and has multiple TATT motifs, one of which is indicative of a cytokine molecule (1). Two polyadenylation signals (AATAAA) were found at 363 and 391 bases upstream from the 3' end. Interestingly the protein lacks the typical sequences indicating cellular secretion. The PBEF protein has six cysteine residues, which suggests that it can act as a zinc finger protein. There are two sites for asparagine glycosylation, four sites for protein kinase C phosphorylation, and five creatine kinase 2 phosphorylation sites. The PBEF protein also has a hydrophobic amino terminus.

In 2001, Ognjanovic (17) and coworkers confirmed and expanded on Samal's original work, finding that the 2.4-kb mRNA encoding PBEF has 11 exons and 10 introns, which is transcribed from the gene spanning 34.7kb on chromosome 7. Exon 1 was shown to encode a short 5' untranslated region (UTR) as well as the signal peptide region. Exon 11 encodes the carboxyl end of the protein product as well as the entire 3' UTR. Ognjanovic's analysis found that the 5' flanking region upstream of the transcription initiation site contains many regulatory elements. The 5' flanking region contains two distinct segments: the proximal 1.4-kb segment and the distal 1.6-kb segment. The proximal segment has a higher percentage of GC nucleotides, whereas the distal segment is more AT rich. The proximal region was found to contain transcription initiation sites, but did not contain TATA or CAAT boxes. However, the distal region did contain several TATA and CAAT boxes, as well as several initiation sites approximately 2 kb upstream of the initiation site. Ognjanovic (17) hypothesized that the distal region could serve as a distal promoter. Binding sites for several transcription factors were identified, including Sp1, NF-1, AP-1, and AP-2. Hormonal responsive and chemical regulatory elements were also found for the glucocorticoid receptor, corticotrophin release factor, cAMP response element binding protein (CREB), and the nuclear factors NF-IL6 and NF-B. The tissue-specific transcription factors liver factor-1 and hepatic nuclear factor were also identified. Comparisons with other proteins revealed that the genomic structure is similar to the human gonadotropin releasing hormone receptor (GNHRH) (17). Both GNHRH and PBEF have a proximal promoter lacking a TATA box, but have a distal promoter with multiple TATA boxes.

In 2000, Martin *et al* identified a gene in *H. ducreyi* that conferred NAD independence (18), and PBEF was found to be the only protein with the same function. This gene was determined to code for the enzyme nicotinamide phosphoribosyltransferase in the NAD biosynthesis pathway. Kitani *et al* (19) characterized the rat version of PBEF and found mRNA variants of 2.3, 2.6, and 4.5 kb in length. The protein was found to be 491 aa residues in length, with a molecular weight of 55.4 kDa. Curiously, while two protein isoforms were detected, it was not determined whether the smaller was a product of protein degradation or alternative splicing. The rat PBEF sequence was found to be 98%, 95%, and 88% homologous to the mouse, human, and carp sequences, respectively, and Kitani speculated that such a highly conserved sequence indicates an important physiological role. McGlothlin (20) and coworkers sequenced the canine version of PBEF, which was found to be 96% identical to human PBEF as well as 94% identical to murine and rat PBEF. The canine cDNA sequence was smaller than the others at 1476 bp, but similar to human and rat, and contains one open reading frame (ORF) encoding a protein of 491 aa. Analysis of this protein predicted two O-glycosylation sites, one N-glycosylation site, 14 serine phosphorylation sites, three threonine phosphorylation, and seven tyrosine phosphorylation sites. These were found to be identical in human, mouse and rat, except at serine 303, threonine 220, and tyrosine 87.

Human PBEF was further investigated by Bae and colleagues (21). Previously, other adipocytokines have been shown to be regulated by hypoxia, so PBEF was investigated for that ability. In breast cancer cells, hypoxia was shown to increase PBEF mRNA and protein levels, and the molecular mechanism was found to involve hypoxia-inducible factor 1 (HIF-1). Further analysis of the genomic region around PBEF found that the 5'-flanking promoter contains two HIF-responsive elements (HREs), and the interaction of these HIF-1 with the HRE sites is responsible for the expression of PBEF.

In 2006, the crystalline structure of mouse PBEF was solved (22), as well as the structure of rat PBEF by itself and in complex with the potent Nampt inhibitor FK866 (23). The PBEF structure was reported to belong to the dimeric class of type II phosphoribosyltransferases, with the active site lying at the dimer interface where the two Nampt molecules bind. In the apoenzyme structure, a sulfate ion binds in place of the phosphate of NMN. A unique aspect of Nampt is the existence of a hydrogen bond between Asp219 and the amide of nicotinamide, which prevents the enzyme from forming a hydrogen bond to nicotinic or quinolinic acid. Other enzymes in the NAD pathway do not contain this feature. It was also predicted that Nampt/PBEF should be able to autophosphorylate, which would be consistent with other type II phosphoribosyltransferases. The porcine version of the PBEF gene was sequenced (24), and three splice variants were found, one occurring also in other species.

Many possible post-translational modifications have been predicted from the genomic structure, but currently no reports have confirmed that they actually occur. Further investigations will need to determine which modifications occur and what their role is in relation to PBEF function.

Localization and regulation of PBEF expression

Tissue distribution and regulation of PBEF

PBEF is found in a variety of tissues and cells. Samal *et al* (1) reported that human PBEF has the highest levels of expression in bone marrow, liver tissue, and muscle cells, but is also expressed in a variety of other tissues, including placenta, kidney, heart, and lung. In 2001, Ognjanovic (17) showed that PBEF was localized in fetal cells from the amnion and chorion, as well as in maternal decidual cells. Kitani (19) investigated the human and rat tissue distribution of PBEF, finding that human PBEF expression is the highest in

leukocytes followed by liver and lung and that there was a similar distribution in rat, except that PBEF expression was higher in heart than in lung.

McGlothlin *et al* (20) were able to produce an antibody for canine PBEF with which they showed that in canine, PBEF is mostly highly expressed in liver followed by muscle cells. In human, McGlothlin was able to show that PBEF is expressed in lung tissue. In 2007, Revollo (6) investigated the tissue distribution of murine intracellular PBEF and reported that the highest levels were found in brown adipose tissue, liver, and kidney. Heart was shown to have an intermediate level, and white adipose tissue, lung, spleen, testis, and muscles had the lowest levels.

Several regulatory elements were found in the genomic structure of the human PBEF gene, with binding sites for NF- κ B, AP-1, and NF- κ B. The presence of cAMP response element binding protein (CREB) and AP-2 binding sites indicated that PBEF could be readily regulated by hormones and phosphorylation pathways, and it was found that tumor necrosis factor (TNF), IL-1, and IL-6 all increase the production of PBEF. Several glucocorticoid responsive elements (GREs) were also found in the promoter region of PBEF, and dexamethasone, a strong glucocorticoid steroid, was found to inhibit the effects of IL-1 and TNF- α on PBEF, which strengthens the hypothesis that PBEF is an inflammatory molecule.

While searching for novel adipocytes, Fukuhara (14) measured mRNA expression in visceral as well as subcutaneous fat in humans and mice, and PBEF was found to have a much higher mRNA expression level in visceral fat than in subcutaneous fat. In 3T3-L1 adipocytes, dexamethasone and growth hormone increased expression of PBEF in a dose- as well as time-dependent manner (25),(26). α -Adrenergic agonists and accumulation of cAMP were also able to downregulate PBEF expression. Kralisch (27) showed that IL-6 downregulates PBEF in 3T3-L1 cells and that it functions partly through the p44/42 MAP kinase pathway. It was also reported that PI3-kinase may play a role in maintaining normal levels of PBEF expression. Segawa (28) *et al* reported that significant upregulation of PBEF expression occurs in hypoxic 3T3-L1 adipocytes. In pre-adipocytes, PBEF expression is decreased by insulin, T3, progesterone, testosterone, palmitate, and oleate, while in differentiated adipocytes, insulin decreased PBEF expression by more than twice as much. Oleate and sex hormones did not have any effect on adipocyte expression of PBEF, but TNF- α and PPAR- α agonists decreased its expression. The inhibitory effects of T3 on PBEF were confirmed in patients with Graves's disease (29) by measuring PBEF levels before and after antithyroid treatment. Before treatment, the PBEF level was 20.7 ng/ml, which is higher than in normal subjects (16.2 ng/ml). Following antithyroid treatment, the serum levels of PBEF dropped to 12.0 ng/ml. The levels of PBEF did not correlate with indicators of insulin resistance.

In contrast with the 3T3-L1 cell culture studies, TNF- α (5.75 nmol/l) increased PBEF expression in human visceral adipose tissue by 255% after 24 hours (30) and by 341% after 72 hours. TNF- α was also found to increase expression of PBEF in monocytes, and this effect was enhanced in the presence of oxLDL (11).

Sub-cellular localization

- Extracellular: Samal (1) *et al* set out to find new cytokines by checking for novel proteins with responsiveness to cycloheximide. PBEF was found using this criterion and was therefore determined to be a cytokine. Ognjanovic (17) strengthened the case for PBEF as an extracellular cytokine by reporting several cytokine transcriptional elements in its genomic structure. Fukuhara (14) found that

PBEF is a secreted factor highly expressed in visceral fat, while Revollo (6) was able to confirm the presence of PBEF in mouse circulating blood.

- **Intracellular:** In 2002, Rongvaux (2) tested some of the immune modulation functions of PBEF, specifically in relation to antibody-mediated immune responses. During the course of these experiments, PBEF was found to be mostly contained in the cytoplasm of spleen cells. Similarly, Revollo (6) found that HIB-1B and 3T3-L1 differentiating cells have a high level of intracellular PBEF. It has also been reported (8) that PBEF is contained in the cytosolic but not in the vesicular fraction of cytoplasm.
- **Nuclear:** Kitani (19) found a higher level of PBEF in the nucleus than in the cytoplasm of proliferating cells, while the converse was true in non-proliferating cells. Kitani was also unable to detect secreted PBEF and postulated that PBEF is not a cytokine.

Many papers provide evidence that PBEF has intracellular as well as extracellular functions. One of the main functions of PBEF is as the Nampt enzyme, which has intracellular activity. However, given the extensive characterization of PBEF as an inflammatory cytokine, an extracellular enzymatic activity cannot be ruled out. It is possible that PBEF is post-translationally modified before it is released from the cells. It is not clear, however, whether PBEF has any function in the nucleus.

Kralisch (25) and MacLaren (26) reported that dexamethasone increases PBEF expression. In several recent reports (6, 14), it was found to have its highest levels in visceral fat and to be expressed at a six-fold higher level in 3T3-L1 cells than in *in vivo* mouse fat tissue. This latter observation could be a clue to whether PBEF is inhibited or increased by dexamethasone, a point that has been disputed in different reports. In cell culture experiments, dexamethasone upregulated PBEF to a level six times above the physiological level. However, when PBEF is already at supraphysiological levels, dexamethasone does not have any further effect on its production. Taking the report of Hector *et al* (30) into consideration, it is also possible that PBEF behaves differently *in vivo* than in *in vitro* cell lines. In addition, it is possible that it behaves differently in adipocytes and endothelial cells.

The relationship between PBEF and other cytokines

Samal *et al* (1) originally reported that PBEF is a cytokine, because PBEF's expression was enhanced by cyclohexamide, and Ognjanovic *et al* (4) conclusively showed that PBEF is indeed a secreted cytokine in amniotic WISH cells. Ognjanovic's subsequent genomic characterization provided more evidence that PBEF is a cytokine, as well as confirming Samal's observation of a lack of a cytokine secretion sequence (17). Specifically, it was noted that PBEF lacked the sequence motif "GPuGPuTTPyCAP", which is normally found on the 5' end of hematopoietic cytokines. However, several important regulatory elements for cytokines, including binding sites for NF- κ B, NF- κ B, and AP-1, were found in the sequence. Also, PBEF has several glucocorticoid regulatory elements that have been shown to inhibit the production of cytokines.

Several papers have argued that PBEF is not a cytokine. Kitani and Rongvaux cited the major function of intracellular PBEF, while Kitani (19) found that PBEF was involved in the cell cycle. Curiously, PBEF was found by accident while working on nerve growth factor 1, a known adipokine (19, 31). Rongvaux (2) found that PBEF protein was expressed more in the cytoplasm than in the extracellular space, and proposed that extracellular PBEF was only found after apoptosis due to the fact that PBEF did not contain either of the known secretion sequences. Fukuhara (14) searched specifically for novel adipokines, and identified PBEF as a novel one. PBEF has been shown to react with a large number of inflammatory and anti-

inflammatory cytokines (5), appearing to have the greatest effect on IL-6 (Fig. 1). PBEF has been shown *in vitro* to be able to upregulate IL-6 at concentrations as low as 5 ng/ml. At 50 ng/ml, PBEF was able to significantly upregulate two other inflammatory cytokines, IL-1 and TNF- α (Fig. 1). At 250 ng/ml and 100 ng/ml PBEF, has been shown to upregulate anti-inflammatory cytokines IL-10 and IL-1Ra (Fig. 1). *In vivo* experiments in mice confirmed PBEF's ability to increase production of IL-6, IL-1, and TNF- α . Ognjanovic (7) previously reported similar *in vitro* results with IL-6, TNF- α , and IL-1. In addition to the other cytokines, IL-8 was also found to be upregulated by PBEF (Fig. 1). The effect of PBEF on IL-8 was confirmed while working in monocyte THP-1 cells(11). While the pathway for secretion of PBEF has yet to be characterized (8), it is known that PBEF secretion is neither Golgi-dependent nor microvesicle-dependent. PBEF was shown to upregulate TGF- β 1, an important cytokine controlling several functions in cell growth, differentiation, and apoptosis in immune cells(32), and has recently been found to affect expression of two other inflammatory cytokines, IL-16 and CCR3 (Fig. 1) (33).

In experiments in chicken, PBEF was found to be expressed primarily in skeletal muscle and not in visceral adipose tissue, and it was therefore postulated that PBEF might actually be a myokine and not a cytokine (34).

Even though there is uncertainty about PBEF's cytokine activity, most papers support the position that it is a cytokine. It has been shown to be responsive to a common cytokine inducer, as well as to contain several common cytokine regulatory elements. It has also been shown to upregulate several prominent inflammatory cytokines: IL-6, TNF- α , IL-1, and, more recently, TGF- β 1 (Fig. 1). The effect of PBEFs on IL-8 is less well known. Although it was reported that PBEF may enhance IL-8, there is not yet enough data to reach a solid conclusion. Moschen (5) showed that, in addition to production of inflammatory cytokines, PBEF at high levels caused production of anti-inflammatory cytokines. While this is seemingly a contradiction, there are several possible explanations. It is possible that PBEF functions as an early mediator of inflammation and, once levels of PBEF rise to a certain point, it switches from a positive feedback loop, to a negative feedback loop with the production of IL-1Ra and IL-10. At 250 ng/ml, PBEF upregulates IL-6 levels (>10,000 pg/ml), but also increases the levels of IL-1Ra ($\approx$ 5000 pg/ml) and IL-10 ($\approx$ 300 pg/ml). While there is little doubt about the inflammatory cytokine activity of PBEF, it is still debatable whether PBEF should be characterized as an adipocytokine. Moreover, there is insufficient data to determine PBEF's inclusion as a myokine, although this is an interesting hypothesis.

Molecular mechanisms mediating PBEF functions

While the work on the extracellular signaling of PBEF has been ongoing for several years, until recently, relatively little work has been done in defining its signal transduction function. Yet while much remains unknown about the molecular functions of PBEF, significant progress has been made.

Fukuhara (14) found that PBEF binds to the insulin receptor, which then activates the phosphatidylinositol 3-kinase (PI3K) pathway and causes phosphorylation of AKT and mitogen-activated protein kinase (MAPK) (Fig. 2). Kim (35) *et al* reported that *in vivo* PBEF caused angiogenesis through the mitogen-activated extracellular signal-regulated kinase 1/2 (ERK 1/2) pathway. Revollo (6) found that to have full intracellular or extracellular enzymatic activity, PBEF must be in the dimeric form. The same group was, however, unable to detect any phosphorylation in the AKT pathway which is the downstream of the insulin receptor. On the other hand, Xie *et al* (36) found that in osteoblasts, regulation of glucose by PBEF involved the use of IR phosphorylation. While Revollo *et al* failed to find any AKT receptor activity, several other groups have found that

PBEF does cause AKT activation. While working in mesangial cells, Song (32) found that PBEF activated AKT pathways as early as 5 minutes after incubation, with the maximal results occurring after 30 minutes. PBEF was also found to upregulate the gene expression of PAI-1 and type 1 collagen, and it has also been reported (37) that PBEF enhances matrix metalloproteinase 2 and 9 (MMPs) levels in human umbilical endothelial cells (HUVECs). In addition, PBEF increases the levels of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2). PBEF accomplishes all these tasks through activation of ERK1/2 and the PI3K/AKT pathways. Consistent with other antiapoptotic reports, PBEF prevents apoptosis in endothelial cells. It was found to increase ICAM-1 and VCAM-1 levels in endothelial cells (38), and induce ICAM and VCAM gene promoter activity through NF- κ B. It was also shown in endothelial cells that NF- κ B activation is increased by PBEF, which was also found to increase production of reactive oxygen species (ROS), partially mediated by NADPH oxidase.

Rongvaux (2) found that PBEF is the mammalian homologue of Nampt, which is the enzyme responsible for the condensation reaction of nicotinamide and 5-phosphoribosyl-1-pyrophosphate into nicotinamide mononucleotide. Ognjanovic(4) reported that PBEF has antiapoptotic effects in amniotic and fibroblasts cells. In sepsis patients, PBEF has been shown to block the apoptotic activity of the enzymes caspase-8 and caspase-3 in human macrophages (39). Li (40) *et al* reported that extracellular PBEF is able to attenuate stress-induced macrophage apoptosis *via* activation of the STAT3 pathway by promoting IL-6 tyrosine phosphorylation of STAT3 (Fig. 1). This was shown to occur *via* an autocrine/paracrine route, independent of IR and Nampt enzymatic activity.

Moschen (5) showed that a plethora of possible molecular pathways are activated by PBEF during cytokine production. Inhibition of p38MAPK stopped all cytokine-enhancing effects by PBEF, while inhibition of MAP2K stopped production of inflammatory cytokines IL-1, IL-6, and TNF- α (Fig. 2); blockage of JNK activity stopped TNF- α production; and PI3K blockage inhibited TNF- α and IL-10 production (Fig.2). Wang (41) found in 2008 that PBEF enhances vascular smooth muscle proliferation via the p38 ERK1/2 pathway, and not through the JNK and PI3K/Akt pathways. In addition, they found that the insulin receptor has no relationship to VSMC proliferation. During a myocardial reperfusion experiments (42), it was found that PBEF works on cardiomyocytes *via* the PI3K and MEK1/2 pathways.

Although there are conflicting reports about PBEF's interaction with AKT, most papers support the hypothesis that PBEF phosphorylates AKT. PBEF has been shown to be involved in GLUT1 transport in mesangial cells, as well as the growth of umbilical and vascular endothelial cells. PBEF activates the MAPK and MEK1/2 pathways, and it is likely that most of its inflammatory actions occur *via* these pathways. The beneficial effects of PBEF in cardiomyocytes may be mediated by activation of the PI3K pathways. Also of interest is the PBEF-induced increase in NF- κ B and the NADPH oxidase-induced ROS, which is a hallmark of vascular diseases such as hypertension.

PBEF and leukocytes

PBEF was named from the observation that it is a pre-B cell colony enhancing factor that amplifies the effects of IL-7 and stem cell factors (1). It has since been linked to CD4⁺ T cells (43) and macrophage cell activation (44), and has also been shown *via* microarray studies to be regulated in T lymphocytes by calcium-dependent signals (Fig. 2) (45). Rongvaux (2) also showed in 2002 that PBEF expression is upregulated in adaptive immune responses.

The work on sepsis patients (39) has shown that PBEF is a mediator of apoptosis in neutrophils, but is instrumental in preventing apoptosis during the inflammatory response.

PBEF mRNA was found in peripheral blood monocytes following inflammatory stimuli, and was also found to be induced by several inflammatory mediators, including LPS, IL-1, and TNF- α .

PBEF was also shown to cause an increase in activity of MMP-9 in the human monocyte cell line THP-1 as well as increased activity of TNF- α and IL-8 levels in peripheral blood mononuclear cells (11). Interestingly, the effects on MMP-9, TNF- α , and IL-8 were abolished when the signaling mechanism for the insulin receptor was blocked. PBEF was also found to have a dose-dependent effect on secretion of TNF- α , IL-1, and IL-6 in CD14⁺ monocytes, but was not able to cause secretion of TNF- α and IL-1 in monocyte-derived macrophages and dendritic cells (5). It was, however, able to increase IL-6 levels in both cells.

PBEF was also shown to enhance the responsiveness of T cells independently of B cell activity and to increase the expression of monocyte cell surface stimulatory molecules CD54, CD40, and CD80, but with no effect on MHC class II, CD69, or CD86(5). PBEF was also found to have dose-dependent chemotactic effects on CD14⁺ monocytes and CD19⁺ B cells, but this effect was not seen in CD3⁺ T cells.

Recently, PBEF has been shown to be vital to the development of T cells and B cells (Fig. 2) (46). Also described in immune cells is the role of PBEF's Nampt activities, which conveys the ability to survive during the oxidative and genotoxic stress environments found in inflammation. The extracellular version of PBEF was shown to stop macrophage apoptosis during stress *via* the STAT3 survival pathway (40).

PBEF has been found to affect virtually all immune cells (Fig. 2). It has been repeatedly shown to be vital to T and B cell development as well as various other activities. PBEF's overall effect is to increase the inflammatory response. A number of cells produce IL-6 in response to PBEF, which increases the levels of CD54 and CD40. CD54 is the marker for ICAM1 (47), which helps in the inflammatory process with leukocyte infiltration, while CD40 (48) is a co-stimulatory marker found on antigen-presenting cells, such as T helper, and B cells, which allows T helper cells to activate B cells. It is interesting to note that while PBEF does increase CD80, it does not increase CD86. CD80 and CD86 are co-stimuli that bind to CD28 (49), and this binding is needed for T cell activation.

PBEF has been shown to increase IL-6 secretion in a variety of lymphocytes. It is possible that, in addition to its role in early inflammation, PBEF helps switch from innate immunity to adaptive immunity. While IL-10 (50) is mainly an inhibitor of cytokines such as IL-1, IL-6, and TNF- α , this effect is caused in large part by inhibition of CD80 and CD86. While IL-10 is also able to affect some naïve CD4⁺ by inhibition of CD28, it can also help stimulate B cell activation, prolong B cell survival, and stimulate proliferation of certain CD8⁺ T cells (Fig. 2). These actions suggest that prolonged secretion of PBEF causes immune cells to switch from innate to adaptive immunity.

PBEF's role as a nicotinamide phosphoribosyltransferase

PBEF functions mainly as a nicotinamide phosphoribosyltransferase (Nampt) (2, 51), which catalyzes the rate limiting step in the mammalian salvage pathway production of nicotinamide adenine dinucleotide (NAD⁺), an important substrate in a number of different reactions, such as sirtuin histone deacetylation (3), ADP-ribose synthesis (52), and NADP synthesis (53).

In mammals, two biosynthesis pathways are known to produce NAD⁺, the *de novo* pathway and the salvage pathway (3). The *de novo* pathway starts with the amino acid tryptophan,

which is converted through a number of steps to nicotinic acid mononucleotide (NAMN) (54), which is converted to nicotinic acid adenine dinucleotide (NAADP), which is then converted to NAD. While there is a *de novo* pathway, the main route of NAD production is thought to be the salvage pathway (51). Nicotinamide is converted by Nampt to nicotinamide mononucleotide (NMN), which is then converted to NAD⁺ *via* nicotinamide mononucleotide adenylyltransferase. NAD can then be converted back to nicotinamide (51) or to NADH or NADP⁺ (53). Silent information regulator 2 (SIR2) is able to convert NAD back to nicotinamide (51). NAD can also be converted to either NADH *via* alcohol dehydrogenase (51) or converted to NADP⁺ *via* NAD kinase (53). NADP⁺ is the oxidized form of NADPH (55). In the process of oxidizing NADP⁺, NADPH oxidase (NOX) produces ROS (56).

Sirtuin family genes have been shown to have many beneficial effects (54). For example, SIR6 and SIR1 detoxify ROS (57), repair DNA (58), and decrease cell sensitivity to apoptosis (59–61). SIR1 has been shown to cause these effects by inhibiting FOXO1, and the DNA repair activity is further mediated by increasing GADD45 (58). Inhibition of FOXO also downregulates the apoptotic factors Fas and Bcl-2 (59, 61) and increases production of superoxide dismutase 2 (57). The intracellular effects of PBEF have been shown to protect cells from damage, and PBEF has therefore been suggested to be a master regulator of aging in mammals (62). However, due to its roles as an inflammatory cytokine and immune modulator, it has yet to be determined whether overexpression of PBEF would be beneficial.

PBEF pathophysiology

In addition to its three distinct physiological roles, PBEF has also been implicated in numerous pathological processes. Due to its roles in inflammation, PBEF has been suggested to play a role in inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, and sepsis. It has also been extensively linked to sepsis-induced acute lung injury and may be involved in cancer through its Nampt activities. FK866 is a well-characterized inhibitor of PBEF that is used primarily as an anticancer drug. PBEF may also be related to cardiovascular disease and type 2 diabetes mellitus by its adipocytokine functions.

PBEF and acute lung injury

PBEF was first shown by Ye to play a role in acute lung injury (ALI) (Fig. 3), with the PBEF level increasing three fold during injury (63). It has also been postulated that PBEF is a receptor for both mechanical stretch and inflammation, and Ye (12) next showed that PBEF is required for completion of the thrombin-induced inflammatory reaction and that PBEF plays a novel role in the regulation of extracellular Ca²⁺-dependent cytoskeleton rearrangement and endothelial dysfunction. PBEF was later shown to interact with NADH dehydrogenase subunit 1 (ND1), ferritin light chain, and interferon-induced transmembrane 3 (IFITM3) in human pulmonary vascular endothelial cells (64). ND1 and IFITM3 have been found to be involved in inflammation and oxidative stress, and its role has also been characterized in ventilator-induced lung injury (VILI) (65) in which it was shown to initiate ALI and VILI gene modules, including NF- κ B, apoptosis, and toll-like receptor pathways. Decreased PBEF was also shown to assuage the severity of VILI. The mechanism by which PBEF enhances ALI may be related to its effects promoting cytokine release (66). It was also found that PBEF increased baseline and TNF- α -mediated IL-8 release by 5 to 10 fold, and also increased cell permeability by more than 30 percent, while knockdown of PBEF significantly lowered the mRNA levels of IL-8.

PBEF and inflammatory diseases

Moschen (5) found that plasma levels of PBEF in Crohn's disease and ulcerative colitis patients are higher than in healthy controls, and its mRNA expression is significantly increased. It is hypothesized that macrophages, dendritic cells, and colonic epithelial cells could be additional sources of PBEF. Gosset *et al* (67) explored the role of PBEF in osteoarthritis (Fig. 2). PBEF was shown to trigger the release of excessive amounts of prostaglandin E₂. PBEF was found to have this action by increasing microsomal PGE synthase 1 (mPGES-1) synthesis and decreasing 15-hydroxyl-PG dehydrogenase (15-PGDH). Under inflammatory conditions, PGE₂ is synthesized by COX2 and mPGES-1 and catabolized in an NAD⁺-dependent reaction by 15-PGDH. PBEF was also found to stimulate release of ADAMTS-4, ADAMTS-5, MMP-3, and MMP-13. PBEF may also contribute to the development and progression of rheumatoid arthritis (RA) (Fig. 2) (10). IL-6 trans-signaling and the IL-6-like cytokine oncostatin M may mediate the role of PBEF in the pathogenesis of RA. In RA patients, PBEF was found to be upregulated by toll-like receptor ligands and cytokines typically found in synovial fibroblasts (68). Synovial fluid levels of PBEF correlated with the degree of inflammation and disease activity, and significantly higher plasma levels of PBEF were found in RA patients (69). PBEF has been found to prevent apoptosis of neutrophils *via* caspase-3 and caspase-8 during clinical sepsis (39), and it was further speculated that PBEF is a mediator of late-phase systemic inflammatory responses.

PBEF and atherosclerosis

It has been found that the PBEF level is increased in the serum of patients with metabolic syndrome or carotid atherosclerosis (Fig. 3) (70). PBEF gene expression is increased in carotid plaques from patients with atherosclerosis (11), and the protein localized to areas containing lipid-rich macrophages. Both ox-LDL and TNF- α induced greater expression of PBEF in monocytes, which was also found in patients with acute myocardial infarction. However, in a recent study of hemodialysis patients with atherosclerosis, PBEF level was not correlated with the atherosclerotic changes, but rather with the inflammatory status of the patient (71). Later studies found that PBEF induces MCP-1 and CCR2 mRNA expression and protein secretion in human endothelial cells (72), and both of these proteins were found to contribute to PBEF's ability to promote angiogenesis.

PBEF and cardiovascular disease

PBEF may be related to cardiovascular disease (Fig. 3). PBEF derived from perivascular adipose tissue was able to stimulate vascular smooth muscle cell (VSMC) proliferation, but did not cause VSMC relaxation (41), and this proliferation was induced *via* ERK1/2 and p38 signaling pathways. PBEF was found to have anti-apoptotic effects on VSMC only during apoptosis caused by H₂O₂, and these effects were found to happen independently of insulin receptor signaling. The proliferation pathways were also found to be mediated by the biosynthesis of NMN *via* PBEF. In other studies (73), PBEF overexpression was found to promote VSMC maturation and to have Nampt activities in SMCs, which are required for their maturation. The formation of new blood vessels from SMCs is dependent on PBEF, and, in addition, PBEF has been found to have cardioprotective effects during times of cardiac ischemia and reperfusion, while reducing myocardial infarct size *in vivo* if administered during reperfusion. PBEF was also found to reduce cardiomyocyte death when administered at the time of reoxygenation (42), and both of these effects function through the PI3K and MEK1/2 pathways. It was thought that the protective activities of PBEF are from inhibition of mPTP pathways in the cardiomyocytes.

PBEF and type 2 diabetes (T2DM)

PBEF may play a role in T2DM (Fig. 3). Fukuhara described PBEF as an adipocytokine that has the ability to bind to the insulin receptor and mimic the effects of insulin (14). Since then, much work dealing with diabetes has concentrated on PBEF. Plasma levels of PBEF were found to be elevated in test subjects with T2DM (74), with levels of 15.8 ± 16.7 ng/ml for normal human subjects and 31.9 ± 31.7 ng/ml for T2DM patients. Increased levels of PBEF were independently and significantly associated with T2DM. PBEF was found in the cerebrospinal fluid (CSF) (75), and CSF PBEF levels were lowered as BMI increased in T2DM patients. It is thought that the ability of PBEF to cross the blood–brain barrier is impaired in obesity and that lower levels of CSF PBEF are involved in obesity pathologies. Later studies found that glucose increases the PBEF level in adipocytes (76), while its level was found to be lowered in morbidly obese subjects following gastric banding procedures (77). Several SNPs of the PBEF gene were found that are thought to determine the susceptibility to T2DM by mediating chronic low-grade inflammatory responses (78). A second SNP study found that the –1535 promoter variant of PBEF gene is associated with lower serum triglyceride and increased HDL cholesterol levels in Japanese patients (79).

PBEF levels were found to increase after gastroplastic surgery (80). Women with gestational diabetes mellitus were found to have increased levels of PBEF during pregnancy, which continued to rise after parturition (81). PBEF, however, showed no association with insulin. In later studies, PBEF levels were found to increase slightly from 15 to 18 ng/ml in T2DM patients, but increased to 37 ng/ml in type 1 diabetes patients (82). Further evidence against PBEF's role in T2DM is the observation that the PBEF protein and mRNA levels are lower in obese patients than in controls (83). Evidence is lacking to support any relationship between PBEF and insulin resistance. Studies of severely obese patients with jejunoileal bypass surgery showed that the PBEF levels were increased only with high glucose levels (84). These patients had much higher levels of PBEF, which were previously reported to be 55.9 ± 39.9 ng/ml. While there are several reports of PBEF upregulation in obesity and T2DM, others contradict this finding. The large volume of contradictory data suggests that there may be a problem with the immunoassays used to detect PBEF in these clinical trials. Researchers investigated this phenomenon in later 2007, and conflicting results were found using different immunoassays (85). The clinical research should be repeated once a reliable assay is found.

Unfortunately, Fukuhara's paper, which reported that PBEF binds to insulin receptors (IR) and activates IR, was later retracted from *Science* (15). Several researchers, including Revollo and Moschen, have not been able to detect PBEF binding to IR (3, 6). Interestingly, Dahl *et al* (11) showed that in peripheral blood mononuclear cells, production of IL-8 and TNF- α was abolished when the insulin receptor was blocked. While it is likely that PBEF does bind to the IR, the picture is unclear as to when and how this happens.

PBEF and kidney disease

In studies of patients with chronic kidney disease (CKD), PBEF levels were found to be affected by the glomerular flow rate, but were not related to insulin resistance (86). PBEF levels, however, were correlated with the levels of VCAM-1, which is a marker of endothelial damage. It has also been suggested that levels of PBEF are increased in mesangial cells by high glucose levels (87). Angiotensin II was not found to increase PBEF levels. Interestingly, high levels of PBEF were reported to cause a rapid uptake of glucose *via* GLUT1, indicating a role for PBEF in diabetic nephropathy (Fig. 3).

Future directions

Nampt, high mobility group box 1 (HMGB1), RAGE, and TLR

NAD, which is converted to NMN by PBEF, serves as a substrate in many ADP ribosylation reactions (55). Mono-ADP-ribosylation reactions (88) have been found to modify high mobility group box 1 (HMGB1) protein (55, 89–90), which has recently been found to have some chemokine effects on mononuclear cells (91) and to be an inflammatory cytokine (89). Like PBEF, HMGB1 lacks a classical cytokine secretion signal (92) and has been found to be elevated in sepsis patients (89). Both cytokines are induced in response to LPS (93). HMGB1 has been found to signal through RAGE and toll-like receptors (TLR) 2 and 4 (92). RAGE receptors react with HMGB1, amyloid- β , and various other ligands (92). HMGB1 and TLR2 have been implicated in rheumatoid arthritis (RA) (92), and HMGB1, along with RAGE, has also been implicated in atherosclerosis (94). In addition, HMGB1 has been shown to play a role in acute lung injury (95–96). Since many of the cytokine effects of HMGB1 are similar to those of PBEF, it will be interesting to investigate the relationship of PBEF to HMGB1, TLR2, TLR4, and RAGE. Even if PBEF and HMGB1 do not directly interact, their effects are almost certainly synergistic. Since RAGE and TLR2 are present in RA, atherosclerosis, sepsis, and ALI, they could be novel mediators of the release of PBEF.

Nampt, NAD, and SIRT

NAD also serves as an essential cofactor of SIRT, a deacetylase (97). The relationship of SIRT1 and PBEF has been reviewed extensively elsewhere (16, 55). Kitani reported PBEF in the brain of rats, and Hallschmid (75) measured levels of PBEF in the CSF. SIRT1 has been shown to protect against microglial-dependent amyloid- β toxicity (98). CD40 is necessary for amyloid-induced microglia activation (99), and PBEF increases CD40 expression. Therefore, it will be interesting to determine whether PBEF plays a role in normal brain physiology as well as in the pathophysiology of Alzheimer's disease.

Immune system and hypertension

While it is well known that atherosclerosis is an inflammatory disease of the circulatory system, hypertension may be directly affected by the immune system (100). In angiotensin II-mediated hypertension, chemokine receptor 2 (CCR2) was found to play an important role in the development of hypertensive nephropathy resulting from increased levels of oxidative stress and inflammation (101). Consistent with this, immunosuppressive treatment with dexamethasone was found to stop angiotensin II-induced renal damage due to suppression of T cell infiltration as well as MHCII and CD86 expression (102). While human T and NK cells were found to possess a functional renin–angiotensin system (RAS) (103), blockade of AT₁ and AT₂ receptors did not alleviate the effects of AngII, leading to the conclusion that other AngII receptors are functional in leukocytes. Using DOCA-salt-induced hypertensive mice, it was shown that AngII increased T cell markers of activation, as well as T cell populations in perivascular adipose tissue, which expressed high levels of chemokine receptor 5 (CCR5). During hypertension, T lymphocytes also produced higher levels of TNF- α , and Harrison *et al* recently found that T cells may play a role in the pathogenesis of hypertension (104). In both T and B cell knockout mouse models, angiotensin II-induced hypertension was attenuated. When T cells were adoptively transferred back into the mouse, angiotensin II-induced hypertension was increased. However, T cells adoptively transferred back that were missing the angiotensin type 1 receptor or a functional NADPH oxidase were not able to cause hypertension (104), and this signaling was found to run through TNF- α . Furthermore, it was shown that T cells have an endogenous RAS system that modulates TNF- α function (105) and is capable of producing angiotensin II.

While no direct relationship between PBEF and hypertension has yet been reported, PBEF does cause many molecular and cellular events associated with this condition. For instance, it has been shown to increase the levels of NF- κ B(38), TNF- α (5, 7, 11), and ROS (38). Furthermore, PBEF's function as Nampt is the rate-limiting step in the salvage pathway that converts nicotinamide to NAD⁺ (2), which is further converted to NADP (53) and finally to NADPH (106), which converts O₂ to superoxide (O₂⁻) (107). Therefore, it will be interesting to determine whether PBEF is involved in hypertension, which may open a new line of inquiry into the role of inflammation in cardiovascular disease.

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Biography

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Dr. Zhongjie Sun is currently a tenured professor and Director of the Robert & Mary Cade Laboratory in the Department of Physiology at the University of Oklahoma Health Sciences Center. He received his MD in 1983 and PhD in 1999. Dr. Sun started his academic career as an Assistant Professor at the University of Florida in 2000. He joined the University of Oklahoma Health Sciences Center as a tenured Associate Professor in 2006. He was promoted to full Professor in 2011. Dr. Sun's research interest is inflammation in diabetes and cardiovascular disease. He has worked in the fields of inflammation, cytokines, diabetes and cardiovascular disease for over 20 years. He found that the cytokine release and macrophage infiltration play critical roles in the development of diabetes and hypertension. Dr. Sun has published extensively in these fields.

Figure 1.

PBEF stimulates expression and release of other cytokines. **PBEF**, pre-B-Cell colony enhancing factor; **IL**, interleukin, **TGF- 1**, transforming growth factor-1 ; **TNF-** , tumor necrosis factor- ; **NFκB**, nuclear factor κB; **STAT3**, signal transducer and activator of transcription 3; **CCR-3**, C-C chemokine receptor type 2;

Figure 2. PBEF activates T cells, B cells and monocytes. **PI3K**, phosphoinositide 3-kinase; **TNF- α** , tumor necrosis factor- α ; MCP-1, monocyte chemotactic protein-1.

Figure 3.

PBEF is involved in the pathogenesis of several diseases. **MCP-1**, monocyte chemotactic protein-1; **CCR-2**, C-C chemokine receptor type 2; **PI3K**, phosphoinositide 3-kinase; **MEK1/2**; mitogen-activated protein kinase kinase 1/2