Article

Banana lectin from Musa paradisiaca is mitogenic for cow and pig PBMC via IL-2 pathway and ELF1

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Abstract: The aim of the study was to gain deeper insights in the potential for polyclonal stimulation of PBMC from cows and pigs with banana lectin (BanLec) from Musa paradisiaca. BanLec induced a marked proliferative response in cow and pig PBMC. The response in pigs was even higher than to Concanavalin A. Molecular processes associated with respective responses were examined with differential proteome analyses. Discovery proteomic experiments was applied to BanLec stimulated PBMC and cellular and secretome responses were analyzed with label free LC-MS/MS. In PBMC, 3955 proteins were identified. After polyclonal stimulation with BanLec, 459 proteins showed significantly changed abundance in PBMC. In respective PBMC secretomes, 2867 proteins were identified with 231 differentially expressed candidates as reaction to BanLec stimulation. The transcription factor `E74 like ETS transcription factor 1 (ELF1)` was solely enriched in BanLec stimulated PBMC. BanLec induced secretion of several immune regulators, amongst them positive regulators of activated T cell proliferation and Jak-STAT signaling pathway. Top changed immune proteins were CD226, CD27, IFNG, IL18, IL2, CXCL10, LAT, ICOS, IL2RA, LAG3 and CD300C. BanLec stimulates PBMC of cows and pigs polyclonally and induces IL2 pathway and further proinflammatory cytokines. Proteomics data are available via ProteomeXchange with identifier PXD027505.

Keywords: PBMC; polyclonal cell stimulation; complement pathway; plant lectin; Musa paradisiaca; banana lectin; BanLec; LC-MS/MS; proteomics, ELF1, IL-2

1. Introduction

Lectins recognize cell-surface carbohydrates with high specificity and are important for many biological processes [1]. Plant lectins are able to induce various immune responses *in vitro* and also *in vivo*.[2]. Mitogenic lectins can promote polyclonal stimulation in PBMC [3,4]. Earlier, we showed that a lectin from the banana Musa paradisiaca highly stimulated *in vitro* proliferation of cow PBMC [5]. A subgroup of cows reacted with hyperstimulation to classical polyclonal stimulants like pokeweed mitogen [6,7], concanavalin A [4], but also to BanLec from Musa paradisiaca [8]. This banana lectin was the first lectin isolated from the large family of Musa [9]. A study on human antibody binding to various foods, unraveled marked binding of IgG4 to banana (Musa paradisiaca) extract [9]. A mannose-binding lectin was subsequently isolated and named BanLec-I [9]. Bananas are a big family, comprising dessert bananas and plantains [10]. Both belong to the big and permanently increasing family of Musaceae [10,11]. Lectin activity is restricted to certain varieties of banana [12]. These banana lectins belong to the mannose-specific jacalin-related lectins [13]. Proliferation induced by Musa acuminata (Del Monte banana) lectin led to the expression of the

cytokines interferon-γ, tumor necrosis factor-α, and interleukin-2 in mouse splenocytes [12]. BanLec from Musa paradisiaca stimulated T cell proliferation in man in presence of IL-2 like Concanavalin A [14,15]. The immune pathway activated by BanLec-I was not identified in these studies [14,15]. BanLec also had strong mitogenic activity towards murine T-cells [16]. Upon stimulation, secreted cytokines differed in intensity and quality in association with genetic background of mice [16]. Whereas C57 BL/6-originated splenocytes reacted with interferon gamma (IFNG) production to stimulation with recombinant BanLec, splenocytes of Balb/c mice produced interleukin (IL)-4 and IL-10 [16]. Recently, BanLec is getting more and more attention because it could be shown that it has the ability to inhibit HIV replication [17-19]. This BanLec-HIV recognition is thought to be mediated through mannose epitopes on high-mannose N-glycans [20]. BanLec is one of the most potent anti-viral lectins, but also recognizes bacteria with high-mannose type structures [20,21]. This makes BanLec very interesting as a very potent anti-infective agent with additional immune modulatory capacities [21-24]. Further information about the intervention with the immune system is needed to understand respective processes.

For several diseases and for basic biomedical research, pigs are used as large animal models [25-30]. Their advantages are close similarities to humans in terms of size, anatomy, diet and metabolism [27,29-31]. Further, the immune system of pigs resembles man for more than 80% of analyzed parameters in contrast to the mouse, with only about 10% resemblance [32]. Analyses of the T cell repertoire in pigs revealed that the main differences between porcine and human T cells are the high frequency of both CD4+CD8α+and TCR-γδ T cells [33]. Besides these differences, porcine T cells closely resemble human T cells validating the use of pigs for biomedical research into T cells [33]. Further investigations will further extend the knowledge about the porcine immune response [33].

Therefore, the aim of our study was to assess the proliferative response of bovine and porcine PBMC after polyclonal *in vitro* stimulation with BanLec from Musa paradisiaca. Additionally, we wanted to get deeper insights into molecular processes accompanying respective immune stimulation by BanLec. Therefore, we undertook differential proteome analyses with control and BanLec activated PBMC.

2. Materials and Methods

2.1. Animals

In this study, PBMC of 148 healthy dairy cows, from age two to nine years old, and PBMC of 36 pigs were analyzed. Blood withdrawal was performed according to the German Animal Welfare Act with permission from the responsible authority (Government of Upper Bavaria), following the ARRIVE guidelines and Directive 2010/63/EU. Approval numbers: ROB-55.2-2532.Vet_03-17-106 and ROB-55.2-2532.Vet_02-19-195.

2.2. PBMC preparation from pigs and cows

The permission from the dairy farm to use the blood samples from their animals for study purpose was obtained. Blood samples from pigs were obtained either at a local slaughterhouse, or from the chair for molecular animal breeding and biotechnology of Badersfeld. Venous whole blood was collected in sodium-heparin (25.000 I.U.) coated tubes and PBMC were prepared as previously described [8,28]. Briefly, cow blood was diluted 1:2 in PBS (NaCl 136.9 mM, Na_2HPO_4 x $2H_2O$ 8.1 mM, KH_2PO_4 1.4 mM and KCl 2.6 mM; pH 7.4) and PBMC were isolated by density gradient centrifugation (23°C, 500 x g, 25 min, brake off) using Pancoll separating solution (PanBiotech, Aidenbach, Germany). PBMC were obtained from intermediate phase, washed twice in PBS, and immediately

used for *in vitro* stimulation. Pig PBMC were isolated by density gradient centrifugation (23°C, 500 x g, 25 min, brake off) using Pancoll separating solution (PanBiotech, Aidenbach, Germany). PBMC were obtained from intermediate phase, washed twice in PBS (NaCl 136.9 mM, Na_2HPO_4 x $2H_2O$ 8.1 mM, KH_2PO_4 1.4 mM and KCl 2.6 mM; pH 7.4), and immediately used for *in vitro* stimulation.

2.3. Polyclonal stimulation of PBMC with mitogens

To assess the proliferative response to Musa paradisiaca lectin (BanLec, L1410; Vector, Germany; pigs with 1 µg/ml, cows with 5 µg/ml) and two control mitogens, eight experiments with a total of 36 pigs (two technical replicates) and 35 independent experiments with a total of 148 cows were performed. In every assay, technical replicates (duplicates or triplicates) were generated for each animal tested. Mean values of technical replicates were then used for further statistical analysis. As positive controls, PBMC were either stimulated by pokeweed mitogen (PWM; Sigma, Taufkirchen, Germany; pigs with 1 µg/ml, cows with 5 µg/ml) or concanavalin A (ConA; Sigma; pigs with 1 µg/ml, cows with 5 µg/ml). After 34h of stimulation, cells were pulsed for 14h with 0.05 mCi/well [methyl-3H]-thymidine (Perkin Elmer, Hamburg, Germany), harvested and counts per minute were measured using a Microbeta (Perkin Elmer). Proliferation rate was expressed as the ratio of 3H-thymidine incorporation by stimulated cells with respect to unstimulated cells.

2.4. Secretome and cell lysate preparations from pigs for differential proteome analysis

For differential proteome analyses, porcine cells were resuspended in RPMI 1640 (PanBiotech) with 1% Penicillin-Streptomycin (PanBiotech), but without serum. Pig PBMC (2 x 10^7 cells for proteome analyses) were then stimulated with 1 µg/ml BanLec at 37°C and 5% CO2 and unstimulated controls were incubated under same conditions. After 48 h, cells were washed twice with PBS, supernatants were discarded and PBMC immediately fractionated before proteomic analysis. Secretome samples were centrifuged (1000 x g) after 48 h of incubation, the supernatants collected and subsequently digested in a filter-aided sample preparation (FASP). For control of proliferation rate of the porcine PBMC, proliferation assays were performed simultaneously. Therefore, PBMC (1 x 10^5 cells/well) were stimulated in triplicates for 48 h with either 1 µg/ml BanLec, ConA or PWM in 96-well plates as described [28]. Cellular lysates of PBMC and secretome samples were digested by a modified FASP protocol as described [34,35]. Briefly, eluates were diluted 1:10 with 0.1 M Tris/HCl pH 8.5 and 100 mM dithiothreitol was added for 30 min at 60°C. After cooling down, UA buffer (8 M urea and 1 M Tris-HCl pH 8.5 diluted in HPLC-grade water) and 300 mM iodoacetamide were added and incubated for 30 min at room temperature in the dark. Eluates were transferred to 30 kD cut-off centrifuge filters (Sartorius, Göttingen, Germany) and washed five times with UA-buffer and two times with ABC buffer (50 mM NH_3HCO_3 diluted in HPLC-grade water). After washing, proteins were subjected to proteolysis for 2 h at room temperature with 0.5 µg Lys C in ABC-buffer followed by addition of 1 µg trypsin and incubation at 37°C overnight. Peptides were collected by centrifugation and acidified with 0.5% trifluoroacetic acid.

2.5. Mass spectrometric analysis and label-free quantification

PBMC and supernatant samples were digested separately by a modified FASP protocol as described [35]. Eluted peptides were analyzed on a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in the data-dependent mode. Approximately 0.5 µg peptides per sample were automatically loaded to the online coupled ultra-high-performance liquid chromatography (UHPLC) system (UltiMate 3000 –RSLCnano System, Thermo Fisher Scientific). A nano trap column was used (300-µm ID

X 5mm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å; LC Packings, Sunnyvale, CA) before separation by reversed phase chromatography (Acquity UHPLC M-Class HSS T3 Column 75 µm ID X 250 mm, 1.8 µm; Waters, Eschborn, Germany) at 40°C. Peptides were eluted from the column at 250 nL/min using increasing ACN concentration (in 0.1% formic acid) from 3% to 41% over a linear 95-min gradient. MS spectra were recorded at a resolution of 60 000 with an AGC target of $3e^6$ and a maximum injection time of 50 ms from 300 to 1500 m/z. From the MS scan, the 15 most abundant peptide ions were selected for fragmentation via HCD with a normalized collision energy of 28, an isolation window of 1.6 m/z, and a dynamic exclusion of 30 s. MS/MS spectra were recorded at a resolution of 15 000 with a AGC target of $1e^5$ and a maximum injection time of 50 ms. Unassigned charges, and charges of +1 and above +8 were excluded from precursor selection.

Data recorded for lysates and secretomes were processed separately. Proteome Discoverer 2.4 software (Thermo Fisher Scientific, Dreieich, Germany; version 2.4.1.15) was used for peptide and protein identification via a database search (Sequest HT search engine) against Ensembl Pig database (Release 75, Sscrofa10.2; 25 859 sequences), considering full tryptic specificity, allowing for up to two missed tryptic cleavage sites, precursor mass tolerance 10 ppm, fragment mass tolerance 0.02 Da. Carbamidomethylation of Cys was set as a static modification. Dynamic modifications included deamidation of Asn and Gln, oxidation of Met; and a combination of Met loss with acetylation on protein N-terminus. Percolator was used for validating peptide spectrum matches and peptides, accepting only the top-scoring hit for each spectrum, and satisfying the cutoff values for FDR <1%, and posterior error probability <0.01. The final list of proteins complied with the strict parsimony principle.

The quantification of proteins, after precursor recalibration, was based on abundance values (intensity) for unique peptides. Abundance values were normalized to the total peptide amount to account for sample load errors. The protein abundances were calculated summing the abundance values for admissible peptides and these abundances were used for ratio calculations. Ratios above 100 fold and below 0.1 fold were combined into these bins. The statistical significance of the ratio change was ascertained employing the approach described [36], which is based on the presumption that we look for expression changes for proteins that are just a few in comparison to the number of total proteins being quantified. The quantification variability of the non-changing "background" proteins can be used to infer which proteins change their expression in a statistically significant manner.

2.6. Data analysis

Significant ($p \leq 0.05$) differentially abundant proteins in mass spectrometric analyses after stimulation with BanLec were further analyzed. Functional enrichment analysis of cellular and secretome data were done with open source software FunRich [37], version 3.1.4, available at http://funrich.org/index.html.
For determination of Gaussian distribution of proliferatiom data, Kolmogorow-Smirnow (KS) test was used. Since analysed data showed normal distribution (KS test was significant; $p < 0.05$), paired t-test was used for statistical analysis. In pathway enrichment analyses, Bonferroni corrected p-values against whole genome as a background were generated. Statistical probabilities were considered significant at $p \leq 0.05$ (= *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***). Kmeans clustering was visualized with open source software String version 11.0, available at https://string-db.org/.

2.7. Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://www.proteomexchange.org/) via the PRIDE [38] partner repository with the dataset identifier PXD027505. The reviewer account details for access to the PRIDE Dataset PXD027505 are: Username: reviewer_pxd027505@ebi.ac.uk, Password: F6l345ps

3. Results

3.1. BanLec significantly stimulates proliferation of bovine PBMC in vitro

Co-incubation of bovine PBMC with BanLec for 48h resulted in a marked proliferative response of PBMC from 148 tested dairy cows. Highly significant proliferation rate was between pokeweed mitogen and ConA (Figure 1). ConA induced an average proliferation factor of 64,93 (SEM ±2,27), PWM 37,01 (SEM ±1,25) and BanLec 55,10 (SEM ±1,91).

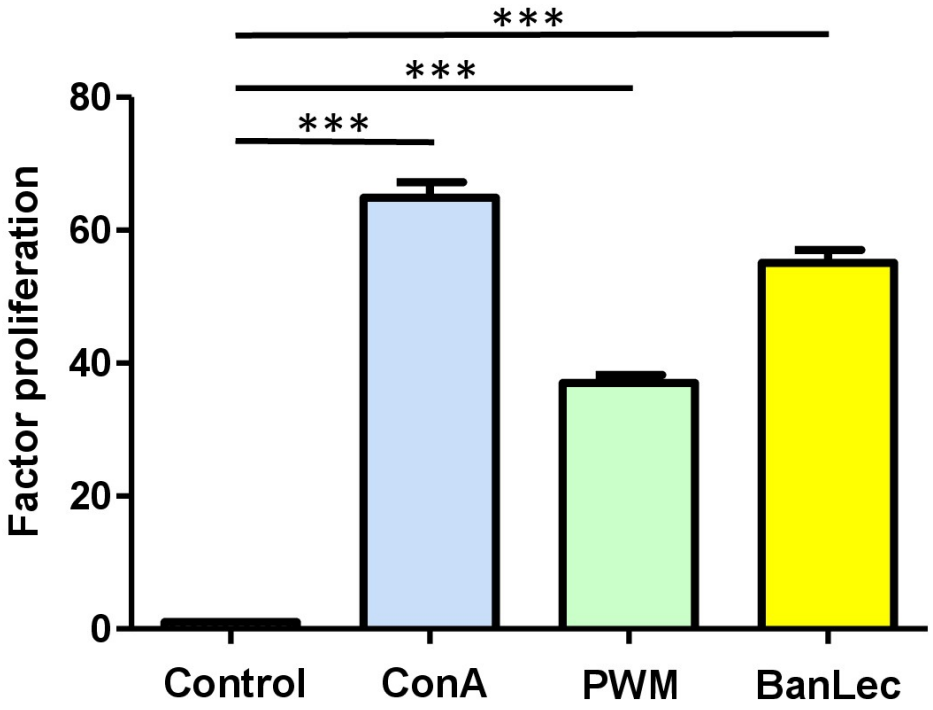

Figure 1: PBMC of 148 dairy cows showed a significantly higher proliferation rate (*** < 0.001) after polyclonal *in vitro* stimulation with ConA (blue bar), PWM (green bar) and BanLec (yellow bar).

3.2. Porcine PBMC proliferate stronger to BanLec stimulation than to ConA

PBMC of pigs also reacted with a significantly increased proliferation rate after polyclonal in vitro stimulation with ConA, PWM and BanLec (Figure 2), with BanLec inducing the highest proliferative response (Figure 2, yellow bar). ConA induced an average proliferation factor of 81,25 (SEM ±9,09), PWM 57,80 (SEM ±5,72) and BanLec 123,80 (SEM ±16,20).

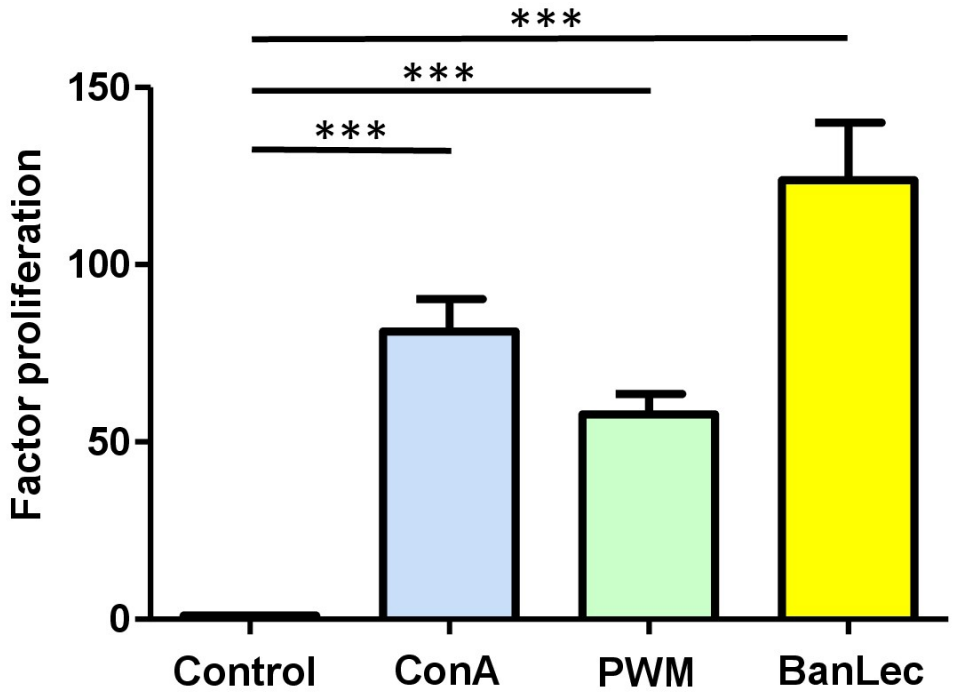

*Figure 2: Porcine PBMC of 36 pigs cows showed significantly higher proliferation rate (*** < 0.001) after polyclonal in vitro stimulation with ConA (blue bar), PWM (green bar) and BanLec (yellow). Ban Lec stimulated PBMC best of the tested polyclonal activators.*

3.3. Significantly changed proteome in BanLec stimulated porcine PBMC

From a total of 3955 proteins identified in porcine PBMC, 459 showed a significantly differential expression. Members from the biological processes 'protein metabolism' (54 mapped proteins) and 'metabolism' (64) were significantly enriched in proteome of BanLec stimulated pig PBMC (table 1 and supplemental tables 1). Cysteine-type peptidase activity' was the only significantly enriched pathway in molecular function. Among the 17 enriched biological pathways, 'cell cycle phases' and 'DNA synthesis' were overrepresented. Protein domains Pept_C1, MCM and DUF4217 were enhanced.

Table 1: Significantly enriched biological processes in BanLec stimulated porcine PBMC.

Biological process	**No. of genes in the dataset**	**Per-centage of genes**	**Fold enrich-ment**	**Bonferroni method**	**Proteins mapped from input data set**
Protein metabolism	54	12,3	1,7	0,02	CBLL1; CLUH; EIF4EBP1; EIF4ENIF1; F8A1; FBXO7; FBXW11; GRSF1; MMP14; MRPL2; MRPL24; MRPL47; MRPL54; MRPS18B; MRPS21; MRPS26; PSMD5; TARS2; TMUB1; UBE2C; USP8; CST7; DTX3L; LGMN; PRTN3; SERPINB2; AGBL3; CUL3; DPEP2; HERC6; ITIH2; MRPL28; RPS15A; ZNRF2; ST6GALNAC2; CTSZ; CTSH; ASNS; HARS2;

Transcription factor	No. of genes in the dataset	Per-centage of genes	Fold enrich-ment	Bonferroni method	Proteins mapped from input data set
ELF1	31	7,7	2,0	0,04	ASAP2; BRI3BP; CDV3; EIF4EBP1; EIF4ENIF1; KLF13; LAG3; LIMS1; MRPL24; NUFIP2; ORC4; RAC1; RGS3; RSRC2; SHOC2; STX5; TBC1D2B; TXNIP; WDHD1; ZMYND8; SERPINB2; SYNE1; BMI1; RCL1; THADA; FCGR2B; ACSL4; UBAP2L; CTSA; HNMT; APOBR
					RPL38; NPM3; ASF1B; ELANE; SELENBP1; CTSA; CTSC; PPIF; CTSB; CTSS; SERPINB10; CELA3A; CSTB; PCNP; MRPS9
Metabolism	64	14,5	1,6	0,04	BCKDHB; COX6A1; CYP51A1; DDX19B; DHCR24; EBP; FAR1; GALT; HCCS; LPGAT1; MAN1A1; MAN2A1; MMAB; NAA40; NUDT15; OXA1L; PDK3; PLCB3; PRMT3; PSAT1; PYCR1; QTRT1; RFK; SOAT1; ACAD9; ADA; ARSA; FUCA2; GAA; GGH; NPL; CHST11; CLPB; DHCR7; PMVK; NAAA; TYMS; IFI30; ACSL4; F13A1; FUCA1; ALOX15; AK4; PAPSS2; MSRA; HEXB; NPC2; SCD; FASN; CA2; MAN2A2; HMGCS1; ASAH1; MTHFD2; CA1; PPT1; PRPSAP1; GALM; HEXA; GALNS; SMPDL3A; ACAD8; TGM3; NAGA

Interestingly, one transcription factor was significantly enriched, this was “E74 like ETS transcription factor 1 (ELF1)”, since 31 from the differentially regulated candidates were associated with ELF1 (table 2 and supplemental tables 1).

Table 2: Significantly enriched transcription factor in BanLec stimulated porcine PBMC.

Biological process	No. of genes in the dataset	Per-centage of genes	Fold enrich-ment	Bonferroni method	Proteins mapped from input data set
Immune response	21	9,7	3,0	0,001	CD226; CD27; IFNG; IL18; IL2; CXCL10; LAT; ICOS; IL2RA; LAG3; CD300C; CD69; C1QB; C1QA; CSF3; AOAH; CFD; C4BPA; CFP; C3; IL1B

3.4. Functionally enriched proteins in secretome of BanLec stimulated PBMC

After polyclonal stimulation with BanLec, we found significant ($p < 0.05$) changes in PBMC secretome of stimulated PBMC. From a total of 2857 proteins identified in secretome of porcine PBMC, 231 were significantly differentially abundant after BanLec stimulation. Functional enrichment analyses of these candidates revealed that proteins from the biological processes 'immune response' (21 mapped proteins) were the top and only significantly enriched biological process in secretome of BanLec stimulated pig PBMC (table 3 and supplemental tables 2).

Table 3: Significantly enriched biological process in secretome of BanLec stimulated porcine PBMC.

3.5. Immune response proteins enriched in BanLec stimulated PBMC cluster to complement activation and Jak-STAT signaling pathway

Kmeans clustering of immune response related, differentially regulated proteins in BanLec secretome resulted in two clusters (Figure 3). One cluster (Figure 3, red dots) comprised proteins related to functions cytokine-cytokine receptor interaction, Jak-STAT signaling pathway and positive regulation of activated T cell proliferation. The second cluster (Figure 3, green dots) included several members of complement cascades.

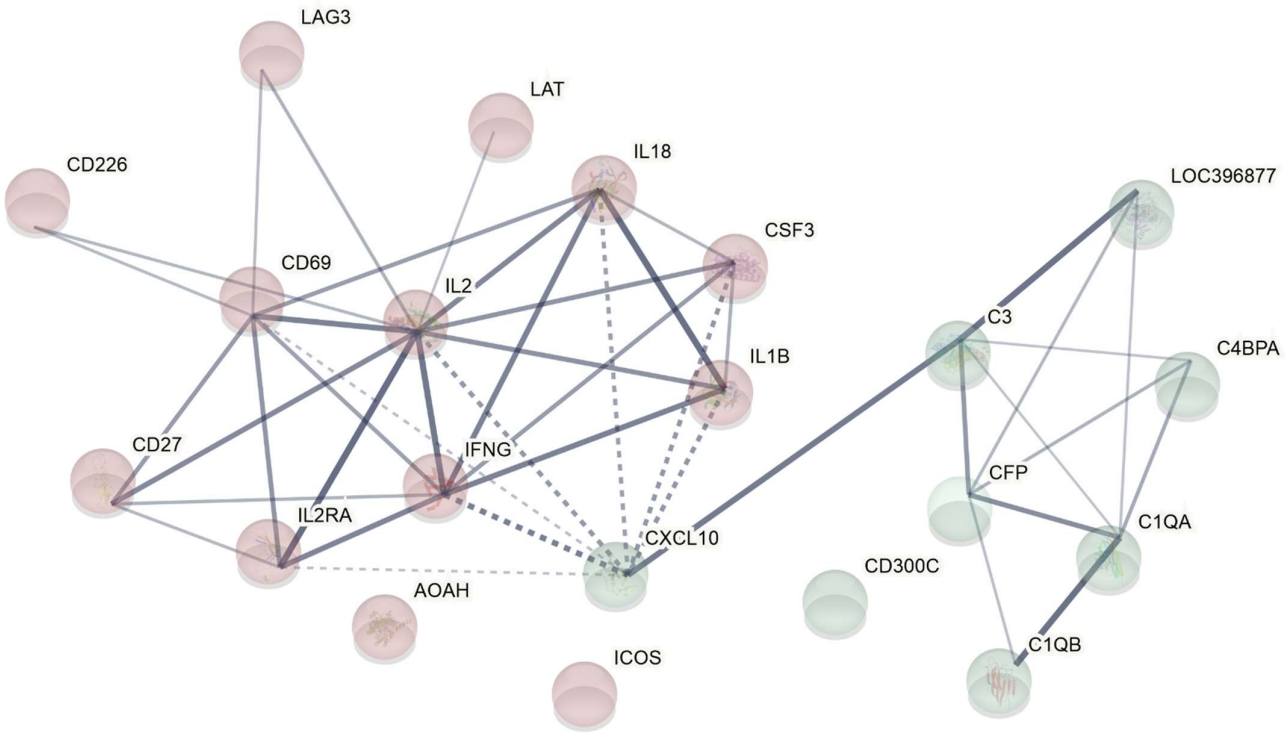

Figure 3: Enriched immune response candidates from PBMC secretome were kmeans clustered, resulting in two clusters (light red and green).

Relations and known interactions of top ten enriched proteins were analyzed with String protein software (version 11, species sus scrofa). For nine proteins, functional relations were found, especially for IL2, IL2RA, IFNG, IL18, CD27 and CXCL10 (Figure 4).

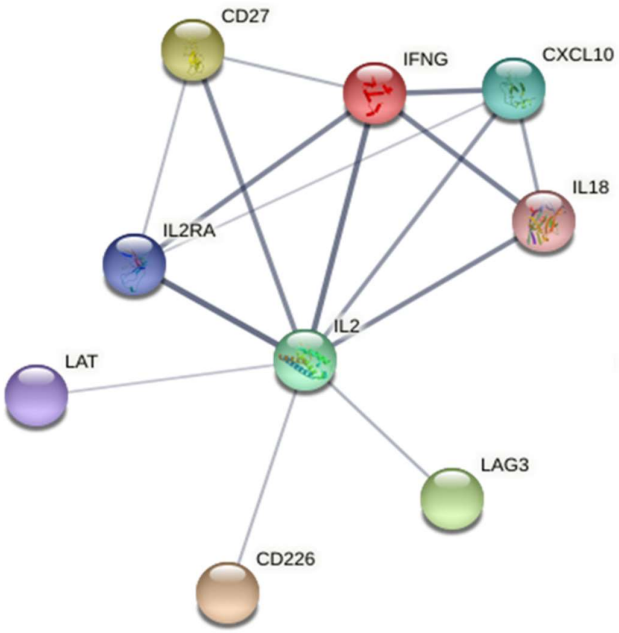

Figure 4: Interaction of top ten enriched immune proteins in BanLec stimulated porcine PBMC secretome.

4. Discussion

BanLec is capable to induce a marked *in vitro* proliferation in bovine and pig PBMC. We observed a difference in overall proliferation capacity between cows and pigs in this study, but we used different concentrations of BanLec for both species, although we used 5 µg/ml for cow and 1 µg/ml for pig PBMC. In pigs, BanLec induced the strongest reaction of all polyclonal activators tested. Therefore, we were interested to detect the immune pathways that were stimulated through BanLec in porcine PBMC. With differential proteome analyses, we detected enhanced proteins that belonged to the biological processes protein metabolism and metabolism, indicating the high rate of reproduction in proliferating PBMC. Analyses of transcription factors surfaced a single factor, ELF1, among the differentially regulated candidates. ELF1 was described as regulator of hematopoiesis and energy metabolism in chromatin immunoprecipitation assays and murine liver cells [39]. ELF1 triggers the NF-κB pathway activation involved in human T leukemic cells [40]. Further, ELF1 is involved in the regulation of several T- and B-cell-specific genes, through the transcriptional activators of BLK and SRC kinases [41]. In pigs, ELF1 was shown to be associated with growth processes [42]. In this study, BanLec activated ELF1 in porcine PBMC. We cannot exactly tell, if BanLec also acted through ELF1 on BLK and SRC kinases from pig PBMC in our study, but we detected BLK and several members of the SRC kinase family in our dataset (detected, unchanged candidates were BLK, SYK, CSK, LYN). A significantly different lower abundant SRC kinase in BanLec stimulated porcine PBMC was Feline Gardner-Rasheed sarcoma viral oncogene homolog (FGR). FGR protein localizes to plasma membrane ruffles, and functions as a negative regulator of cell migration and adhesion triggered by the beta-2 integrin signal transduction pathway in man [43]. The exact function in the immune response of pigs was not described so far.

Significantly higher abundant members of SRC family kinases were Lymphocyte-specific protein tyrosine kinase (LCK) and FYN oncogene related to SRC, FGR, YES (FYN) in secretome of BanLec stimulated porcine PBMC. LCK, a key signaling molecule in the

selection and maturation of developing CD4+ T-cells [44,45] was already shown to increase in porcine PBMC after stimulation with lipopolysaccharide (LPS) by genome sequencing [46]. In our study, LCK was significantly increased (3.2 fold) in BanLec stimulated PBMC. Additionally, FYN was also significantly higher abundant (3,3 fold) in BanLec activated pig PBMC. The function of FYN, an interactor of LYN and inductor of T-cell differentiation and proliferation following T-cell receptor (TCR) stimulation [45], is unknown in pigs so far. In man, FYN inhibits differentiation to Th17 cells [47]. In our opinion, the role of LCK, FYN and ELF1 in pig PBMC deserves further in-depth investigations in the future to clarify their interactions and detailed functions.

The top ten enriched immune proteins in BanLec stimulated PBMC secretome consisted of CD226, CD27, IFNG, IL18, IL2, CXCL10, LAT, ICOS, IL2RA and LAG3.

CD226 is a member of the immunoglobulin superfamily [8]. CD226 is an adhesion molecule that was shown to promote migration, activation, proliferation, differentiation, and function of CD8 + T cells of mice and man [8]. Upon ligand binding, it stimulates T-cell proliferation and cytokine production in humans, including IL2, IL5, IL10, IL13, and IFNG [9]. CD226 is also expressed in memory CD4 + T cells in man [10]. These abnormal cells and relative pro-inflammatory cytokines contribute to many immunological diseases [11-13]. The exact function of CD226 in porcine immune cells was not described so far.

CD27 is a well characterized molecule in pig immune cells [48,49]. CD27 was expressed by all naïve CD8α- T helper cells but divided CD8α+ T helper cells into a CD27+ and a CD27- subset [49]. Analyses with polyclonally stimulated PBMC showed that CD8α+CD27+ T helper cells had an intermediate proliferation rate similar to naïve CD8α-CD27+ T helper cells and also intermediate levels of cytokine production [49]. Therefore, this subpopulation of CD8α+CD27+ T helper cells displayed a phenotype and functional properties of central memory cell like found in humans [49].

IFNG, IL18 and IL2 are proinflammatory cytokines, produced by activated porcine T-cells [49-51]. In the presence of IL18, pigs' lymphocytes produce more IFNG [50]. IFNG and IL18 are pro-inflammatory cytokines in pigs [52] IL18 is a member of IL-1 cytokine family and plays a protective role in many virus infections [52]. It augments IFNG production in T-cells. IFNG activates effector immune cells and enhance antigen presentation [53]. IL2 is important for T cell proliferation in pigs [51]. Additionally, the interleukin 2 (IL2) receptor alpha (IL2RA) was elevated in our study. IL2RA and IL2RB chains, together with the common gamma chain IL2RG, constitute the high-affinity IL2 receptor. A targeted disruption of the X-linked interleukin-2 receptor gamma chain gene in pigs enabled the generation of immune deficient pigs to study the impact of IL2RG for the pig immune response [54]. Only heterozygous pigs survived birth [54]. Il2rg−/Y males had undetectable thymi and reduced peripheral blood T cells [54]. Another study confirmed the severely impaired immune phenotype of Il2rg−/Y pigs with lymphopenia, lymphoid organ atrophy, poor immunoglobulin function, and T- and NK-cell deficiency, underscoring the importance of the IL2 pathway for the immune response [55].

Chemokine (C-X-C motif) ligand 10 (CXCL10) was significantly upregulated as response to BanLec stimulation. In porcine PBMC. CXCL10 is a pro-inflammatory cytokine that is involved in a wide variety of processes such as chemotaxis, differentiation and activation of peripheral immune cell [56]. CXCL10 increased in PRRSV infected pig lungs or after stimulation with poly (I:C) [56].

The linker for activation of T cells was shown to be required for TCR-mediated signaling. LAT interacts in a feedback loop with Zap70 and Src-family kinases [57]. Src-family kinases, e.g. LCK bind phosphorylated LAT [57]. Since we also detected LCK as

upregulated in BanLec activated PBMC, this points to activation of this axis in pig PBMC.

With the inducible T-cell co-stimulator ICOS as higher abundant immune response protein in BanLec stimulated PBMC, we identified an important costimulatory molecule for T-cell proliferation and cytokine secretion [58].

Lymphocyte activation gene 3 (LAG3) belongs to Ig superfamily and is an inhibitory receptor on antigen activated T-cells [59]. Following TCR engagement, LAG3 associates with CD3-TCR in the immunological synapse and directly inhibits T-cell activation [60]. It also mediates immune tolerance through constitutive expression on a subset of regulatory T-cells and contribution to their suppressive function [60]. Its upregulation could point to activation of homeostatic mechanisms in activated pig lymphocytes in order to terminate the inflammatory response through LAG3. Neither LAT, nor ICOS or LAG3 functions in PBMC of pigs are understood to date, therefore, further experiments are needed to clarify their respective roles in the immune response.

5. Conclusions

Lectin from Musa paradisiaca (BanLec) is highly mitogenic for bovine or porcine PBMC. With differential proteome analyses we detected regulated proteins in response to activation by BanLec in pig PBMC. Several immune-related proteins were detected with significantly different abundance in BanLec stimulated PBMC, pointing to a role of the IL2-pathway, CD226, CD27, LAT, ICOS and LAG3. Further, our study points to an important role of the transcription factor ELF1 in BanLec mediated polyclonal stimulation of pig PBMC. BanLec is a very interesting lectin, because it has powerful antiviral properties. But it has also the property to overstimulate the immune system, resulting in harmful side effects that could make it useless as a drug. Here we provide insights into the immune responses activated by BanLec in pigs, an important model organism with high translational value to humans because of the close similarities in immune systems.

Supplementary Materials: Supplemental table S1: FunRich analyses of proteins from cellular dataset, supplemental table S2: FunRich analyses of proteins from secretome dataset.

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Data Availability Statement: The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://www.proteomexchange.org/) via the PRIDE [38] partner repository with the dataset identifier PXD027505.

The reviewer account details for access to the PRIDE Dataset PXD027505 are: Username: reviewer_pxd027505@ebi.ac.uk, Password: F6l345ps

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