Theses of the Doctoral (Ph.D.) Dissertation

Effects of PACAP Deficiency in Systemic Senile Amyloidosis and Peyer's Patches During Aging

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Introduction

Amyloidosis

Amyloid is an abnormal protein aggregate resulting from the misfolding of proteins, and more than thirty different fibril-forming proteins may be responsible for its formation. Fibrillar deposits bind proteoglycans, glycosaminoglycans, and plasma proteins. The most reliable method for detecting amyloid is Congo red staining, which under polarized light microscopy reveals characteristic apple-green birefringence.

Amyloidosis refers to a group of disorders in which the extracellular deposition of amyloid impairs cellular function and can lead to organ failure. It is classified into two major categories: systemic and localized amyloidosis. The systemic form affects multiple organs, while the localized form is restricted to a specific organ. Localized types include, for instance, Langerhans islet amyloidosis associated with type 2 diabetes, and Alzheimer's disease, where beta-amyloid plaque accumulation in the brain leads to symptoms. There are four main types of systemic amyloidosis: primary amyloidosis (associated with monoclonal plasma cell proliferation), secondary amyloidosis (related to chronic inflammatory conditions), hereditary amyloidosis (resulting from genetic mutations leading to transthyretin deposition), and dialysis-related amyloidosis (a consequence of long-term hemodialysis). A special subgroup is senile amyloidosis, which typically occurs in advanced age and may present in either localized or systemic form. In humans, the systemic form most commonly develops around the age of 80, whereas in mice it typically appears at approximately 1.5 years of age. Although its exact mechanism is not fully understood, it is most closely associated with the pathological accumulation of transthyretin protein.

Peyer's Patches and the Immune System

The mucosa-associated lymphoid tissue (MALT) plays a key role in the function of the immune system. A major component of this system is the gut-associated lymphoid tissue (GALT), which includes Peyer's patches (PP), isolated lymphoid follicles, and the vermiform appendix. Peyer's patches are located on the antimesenteric side of the small intestine and consist of clusters of transmucosal lymphoid follicles, appearing as ellipsoid protrusions on the serosal intestinal surface. In humans, there are approximately 100–240 Peyer's patches, found

exclusively in the ileum, while in mice there are typically 5–14, which can be distributed along the entire small intestine.

Histologically, Peyer's patches can be divided into four main regions: (1) the follicle-associated epithelium (FAE) within the epithelial layer; (2) the subepithelial dome (SED) in the lamina propria; (3) B-cell follicles with germinal centers (GC), mantle zones (cortex), and marginal zones (corona); and (4) an interfollicular T-cell zone containing high endothelial venules (HEVs). M cells play a critical role in antigen transcytosis, initiating immune responses that lead to IgA production.

Possible connections between Peyer's patches and autoimmune diseases have long been hypothesized. In Crohn's disease, aphthoid lesions more commonly occur in the terminal ileum. In ulcerative colitis, endoscopic studies have shown that Peyer's patches often display significantly altered morphology, which has been associated with an increased risk of relapse. Additionally, the gut microbiota, through interactions with Peyer's patches, may play a pivotal role in the development of certain autoimmune diseases.

Immune checkpoint molecules expressed on the surface of immune cells play a key role in cancer therapies and personalized medicine. These molecules fall into two main categories: inhibitory (e.g., TIM-3, PD-1) and stimulatory types, which regulate immune responses through interactions with their ligands (e.g., Galectin-9, PD-L1). Immune checkpoint molecules are essential for maintaining immune tolerance, preventing autoimmune processes, defending against infections, and modulating the recognition and elimination of tumor cells. Within GALT, they are especially important for modulating local immune responses and indirectly influencing the composition and balance of the gut microbiota. Experimental studies have demonstrated that in the absence of PD-1, the regulation of T cells within Peyer's patches is impaired, which may be associated with disrupted IgA production. This dysregulation can contribute to the development of dysbiosis. Furthermore, during intestinal ischemia-reperfusion, a decrease in PD-1/PD-L1 expression weakens immune defense mechanisms. CD69, an early activation marker on immune cells, is involved in regulating immune responses in various pathological conditions, including autoimmune diseases, chronic infections, and cancers. In cytotoxic immune cells, perforin and granzyme B are released upon the formation of the immunological synapse, inducing apoptosis in target cells. Elevated levels of these cytotoxic molecules have been implicated in the pathogenesis of autoimmune skin diseases, rheumatoid arthritis, type 1 diabetes, and transplant rejection.

PACAP and the Effects of Its Deficiency in Amyloidosis and on Peyer's Patches

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that increases intracellular cAMP levels via adenylate cyclase activation. It has two biologically active forms, PACAP-27 and PACAP-38, the latter being dominant. Its highly conserved structure across species underscores its essential physiological role. PACAP acts through three G protein-coupled receptors: PAC1, VPAC1, and VPAC2. PAC1 is the most specific to PACAP, while VPAC receptors also bind vasoactive intestinal peptide (VIP). These receptors are widely expressed, especially in the nervous, endocrine, and immune systems. PACAP is involved in numerous physiological functions, including neural, immune, and metabolic regulation. Beyond its neuroprotective, antioxidant, and anti-apoptotic properties, it also supports immune homeostasis, modulates inflammation, and regulates stress responses.

Studies using homozygous PACAP knockout mice have shown that the absence of this neuropeptide leads to various abnormalities, including memory impairment, reduced fertility, increased oxidative stress, and enhanced inflammatory responses. These animals are more susceptible to environmental stressors and have a shortened lifespan. Notably, even partial PACAP deficiency in heterozygous knockout mice or complete PAC1 receptor deficiency can result in severe physiological consequences.

PACAP also plays a significant role in aging, as its declining levels are associated with impairments in cognitive function, vascular regulation, and immune responses. Currently, limited information is available on the relationship between PACAP and amyloidosis. However, our observations indicate that mice lacking endogenous PACAP exhibit early-onset systemic amyloidosis across multiple organs. These findings suggest that PACAP-deficient animals may serve as a model for accelerated aging. Research has shown that PACAP can reduce beta-amyloid deposition in Alzheimer's disease, though the link between endogenous PACAP and systemic amyloidosis remains unclear.

In the gastrointestinal system, PACAP regulates blood flow, intestinal motility, and hormone secretion. Both endogenous and exogenous PACAP have shown beneficial effects in various gastrointestinal conditions, including colitis, ileitis, peritonitis, colorectal carcinoma, and Crohn's disease. However, the literature does not yet provide evidence regarding whether PACAP deficiency affects the macroscopic or microscopic structure of Peyer's patches, the distribution of immune cells within them, or their activation status.

Aims

Previous findings have demonstrated that PACAP levels gradually decline with age, contributing to increased vulnerability of cells, tissues, and organs. Our research group has reported that complete PACAP deficiency in knockout mice leads to accelerated aging and the development of senile systemic amyloidosis, resulting in multi-organ failure, which likely accounts for the increased mortality observed in these animals.

(I) The aim of our first experiment was to investigate whether partial PACAP deficiency in heterozygous PACAP mice, as well as the partial reduction of PACAP signaling in PAC1 receptor knockout mice, contributes to the earlier onset of systemic degenerative processes. To this extent, we compared mice of different age groups and genetic backgrounds to better understand the role of endogenous PACAP in aging. Given that in humans, PACAP levels typically decline partially rather than completely with age, this research carries strong translational relevance.

It has been established that immune cells express PACAP and its receptors, and the neuropeptide exerts anti-inflammatory effects within the body. A hallmark of aging is the structural and functional remodeling of the immune system, manifested by weakened defense against infections and reduced vaccine responsiveness.

(II) In our second experiment, we sought to determine how PACAP deficiency affects gut-associated lymphoid tissue (GALT), with particular focus on the distribution of immune cells and the expression of immune checkpoint molecules within Peyer's patches (PPs) across different age groups. The aim of this investigation was to gain deeper insight into the regulatory role of PACAP on the immune system—especially in the context of aging—and to enhance our understanding of gastrointestinal immune pathomechanisms.

Experiment I – Investigation of PACAP Deficiency in Systemic Amyloidosis

Materials and Methods

In our experiment, we used CD1 background wild-type (WT), homozygous PACAP gene-deficient (KO), and heterozygous PACAP gene-deficient (HZ) mice, as well as WT (PAC1-R WT) and homozygous PAC1 receptor gene-deficient (PAC1-R KO) mice on a C57BL/6J background. For histological analysis, we examined PACAP WT (n = 8), PACAP HZ (n = 10), PAC1-R WT (n = 9), and PAC1-R KO (n = 3) mice. These animals were divided into two age groups: young (3–12 months) and aging (13–24 months). For blood analysis, we first used young and aging PACAP WT and PACAP KO mice (n = 7 per group), followed by 3.5-month-old PACAP WT (n = 11) and PACAP HZ (n = 11) mice. Animals were maintained on a 12-hour light/dark cycle with ad libitum access to food and water. All procedures involving animals were conducted in accordance with institutional protocols and ethical guidelines (ethical approval numbers: BA02/2000-24/2011; BA02/2000-20/2006). Genotyping was performed using the PCR method.

For histological examinations, animals were euthanized using isoflurane overdose, and their organs were fixed in 4% buffered paraformaldehyde and embedded in paraffin. Sections of 3 μm thickness were stained with hematoxylin-eosin and Congo red, the latter used to identify amyloid deposits under polarized light microscopy. Amyloid presence was assessed using a standardized amyloid index ranging from 0 to 3: 0: no amyloid deposition, 1: slight focal; 2: moderate/severe focal or slight diffuse; 3: massive diffuse amyloid deposition throughout the section.

For blood analysis, animals were euthanized with 3% isoflurane, and blood was collected directly from the heart using winged infusion sets into BD Vacutainer tubes. For serum analysis, sodium heparin tubes were used; for routine complete blood count, EDTA-coated tubes were applied. Serum levels of Na^+ and K^+ ions, alkaline phosphatase (ALP), creatinine, cholesterol, triglycerides, and high/low-density lipoproteins (HDL, LDL) were measured using a COBAS 8000 analyzer. Complete blood counts were performed using a Sysmex XN-1000-V Multispecies Hematology Analyzer.

Data were analyzed using appropriate statistical methods. Histological results were evaluated with Mann–Whitney and Kruskal–Wallis tests. Two-way ANOVA was used for blood sample analysis, with Fisher's post hoc test applied to serum parameters and Bonferroni post hoc test to hematological data. A p-value ≤ 0.05 was considered statistically significant.

Results

Histological analyses revealed that partial PACAP deficiency (in PACAP HZ mice) was sufficient to promote increased amyloid deposition, similarly to the condition observed in complete PACAP deficiency (PACAP KO mice). In PACAP HZ mice, amyloid deposits appeared at a younger age and to a greater extent, particularly in the spleen, kidney, esophagus, gastrointestinal tract, and skin. In the spleen, amyloid accumulated in both the white and red pulp regions; in the kidney, it formed homogenous eosinophilic masses within the glomeruli; while in the connective tissue of the esophagus and intestines, diffuse deposits were observed. In the skin, amyloid predominantly accumulated around hair follicles, sebaceous glands, and blood vessels, leading to localized disruptions in blood supply and structural abnormalities. In contrast, PAC1 receptor deficiency (PAC1-R KO mice) did not result in amyloid deposition at either young or advanced ages, suggesting that receptor deficiency alone is not sufficient to trigger amyloidosis.

Serum analysis showed a significant increase in serum creatinine levels in PACAP KO mice, indicating impaired renal function. Lipid parameters exhibited only mild elevations in these animals. In PACAP HZ mice, levels of LDL, HDL, and total cholesterol were significantly higher compared to their WT counterparts, while creatinine levels remained unchanged. Complete blood count analysis revealed no significant differences between WT and PACAP KO mice. However, PACAP HZ mice showed notable changes compared to WT animals. Among the red blood cell parameters, significant differences were observed in the mean corpuscular hemoglobin concentration (MCHC), red cell distribution width—standard deviation (RDW-SD), red cell distribution width—coefficient of variation (RDW-CV), nucleated red blood cells (NRBC), reticulocyte count (RET), as well as in reticulocyte median fluorescence (MFR) and reticulocyte hemoglobin equivalent (RET-HE). Among the platelet parameters, changes were observed in plateletcrit (PCT) values.

Experiment II – Investigation of PACAP Deficiency in Peyer's Patches During Aging

Materials and Methods

In this experiment, we used male wild-type (WT) and homozygous PACAP gene-deficient (KO) mice on a CD1 background. The animals were divided into four experimental groups: young WT (3 months old, n = 10), young PACAP KO (3 months old, n = 14), aging WT (12–15 months old, n = 10), and aging PACAP KO (12–15 months old, n = 18). Animals were housed under controlled conditions and fed a standard laboratory diet, in compliance with ethical regulations for animal experimentation (ethical approval number: BA/73/00452-6/2023). Genotyping was performed using the PCR method.

Young and aging WT and PACAP KO male mice (n = 10 per group) were euthanized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg). Following anesthesia, the abdominal cavity was opened and the gastrointestinal tract was removed. The number of Peyer's patches was counted, and the length of the small intestine, the size of the patches, and the distances between adjacent patches were measured. The stomach and colon were then also removed. Detailed analysis was performed on the number, size, and distribution of PPs, which were subsequently isolated. One PP per animal was processed for histology, while the remaining patches were used for flow cytometric analysis.

For histological analysis, one PP per animal was fixed in 4% buffered paraformaldehyde and embedded in paraffin. Sections of 3 μm thickness were prepared and stained with hematoxylin-eosin (HE). The stained sections were then subjected to qualitative evaluation.

To isolate immune cells from Peyer's patches, we used a mechanical dissociation method to minimize cell loss. Fluorochrome-conjugated monoclonal antibodies were used to label surface proteins, and the labeled cells were analyzed using a FACS Canto II flow cytometer. For intracellular labeling of perforin and granzyme B, cells were permeabilized and stained with specific antibodies. Following fixation and storage, flow cytometric analysis was performed.

For statistical analysis, two-way ANOVA with multiple pairwise comparisons was used to evaluate the effects of age and genotype on the measured immunological parameters. Partial eta-squared values were calculated to indicate effect sizes for statistically significant results. Pearson's correlation coefficient was used to assess linear correlations between immunological parameters. A p-value of less than 0.05 was considered statistically significant.

Results

Macroscopic examination of Peyer's patches (PPs) revealed that their average number was higher in young WT mice compared to older WT animals. In contrast, PACAP KO mice showed similar numbers of PPs regardless of age. No significant difference in PP numbers was observed between aging WT and PACAP KO groups. The average number of Peyer's patches was significantly reduced in aging WT mice compared to young WT mice, and similarly lower in young PACAP KO mice relative to their WT counterparts. Measurements of the distance between adjacent PPs showed no regular pattern across groups; PPs were distributed randomly along the small intestine. The size of Peyer's patches ranged between 2–4 mm in all groups. In aging PACAP KO mice, PPs showed a trend toward reduced thickness and were less prominent on the serosal surface of the intestinal wall compared to other groups.

Histological evaluation of the Peyer's patches revealed no major qualitative or structural differences between WT and PACAP KO mice in either the young or aging groups.

Based on the gating strategy applied during flow cytometric analysis, $CD3^+$, $CD8^+$, $CD4^+$, and $CD4^+/CD8^+$ T-lymphocyte subpopulations were identified, and their frequencies were compared across the different experimental groups. In aging PACAP KO mice, the proportion of $CD3^+$ T cells was significantly decreased compared to young PACAP KO mice. $CD8^+$ T-cell frequency was significantly reduced in aging WT mice compared to young WT mice. $CD4^+$ T-cell levels were significantly lower in aging PACAP KO mice compared to aging WT controls. Within the $CD3^+$ T-cell population, $CD8^+$ T-cell ratios were significantly lower in aging WT mice relative to young WT animals, while $CD4^+$ T-cell levels were significantly lower in aging PACAP KO mice compared to aging WT mice. Interestingly, the proportion of $CD4^+$ T cells relative to $CD3^+$ cells was significantly increased in aging WT mice compared to their younger counterparts. In aging PACAP KO mice, the $CD8^+/CD3^+$ T-cell ratio was significantly reduced compared to young PACAP KO mice but was significantly higher in young PACAP KO mice compared to young WT controls.

Regarding immune checkpoint molecules, PD-1 expression on $CD3^+$ and $CD4^+$ T cells was significantly reduced in aging PACAP KO mice compared to both aging WT mice and young PACAP KO mice. Similarly, PD-1 expression on $CD8^+$ T cells was significantly decreased in aging PACAP KO mice versus young PACAP KO mice. PD-1 expression in $CD4^+/CD8^+$ T cells was significantly lower in young PACAP KO mice than in young WT mice, but significantly increased in aging PACAP KO mice compared to aging WT mice. In aging WT mice, PD-1 expression in $CD4^+/CD8^+$ T cells was significantly lower compared to young

WT animals. PD-L1 expression was significantly reduced in all three T-cell subpopulations ($CD3^+$, $CD4^+$, and $CD8^+$) in aging PACAP KO mice relative to young PACAP KO mice. However, no significant differences in PD-L1 expression were observed between groups in the $CD4^+/CD8^+$ T-cell subset.

Expression of the immune checkpoint molecule TIM-3 was significantly reduced across all T-cell subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$) in aging PACAP KO mice compared to aging WT controls. Conversely, TIM-3 expression was significantly higher in aging WT mice compared to young WT mice, and also increased in aging PACAP KO mice relative to their younger counterparts. Galectin-9 expression was significantly decreased in all T-cell subtypes in aging WT mice compared to young WT controls, as well as in aging PACAP KO mice compared to young PACAP KO mice.

Regarding cytotoxic intracellular molecules, perforin expression in $CD3^+$ and $CD8^+$ T cells was significantly higher in young PACAP KO mice compared to young WT mice. Granzyme B expression in $CD3^+$ T cells was significantly decreased in aging PACAP KO mice compared to aging WT controls. It was significantly higher in aging WT mice relative to young WT mice, and also significantly elevated in aging PACAP KO mice compared to young PACAP KO animals. Similarly, granzyme B expression in $CD8^+$ T cells was significantly higher in aging WT mice compared to young WT mice, and also increased in aging PACAP KO mice compared to their younger counterparts.

Analysis of the activation marker CD69 revealed significantly reduced expression in $CD3^+$, $CD4^+$, and $CD8^+$ T cells in aging WT mice relative to young WT animals, and in aging PACAP KO mice compared to young PACAP KO mice. In contrast, CD69 expression in $CD4^+/CD8^+$ T cells was significantly higher in aging PACAP KO mice than in aging WT mice. However, expression levels were significantly reduced in both aging WT and aging PACAP KO mice compared to their respective younger groups.

Discussion

Advancements in modern medicine and the global improvement in living conditions have contributed to the emergence of aging societies worldwide. Aging and its associated diseases present significant social and economic challenges for humanity. In our experiments, we sought to explore how PACAP deficiency, in combination with aging, contributes to the progression of systemic senile amyloidosis and affects the morphological and functional alterations of Peyer's patches, key components of the enteric immune system.

In our first experiment, we initially examined the effects of PACAP's specific receptor deficiency using PAC1 receptor knockout mice. Our histological observations showed no signs of amyloidosis in these animals. While the PAC1 receptor is critical in many physiological processes, the body may activate compensatory mechanisms in its absence.

In contrast, experiments with PACAP-deficient mice revealed significant pathological changes, particularly in relation to aging, including early onset and more severe progression of systemic amyloidosis. The most pronounced changes associated with PACAP deficiency were observed in the spleen, kidney, gastrointestinal tract, and skin. These results support the idea that PACAP plays a protective role in preventing and slowing aging-related degenerative processes.

Complete PACAP deficiency leads to multiple pathological outcomes, including increased stress sensitivity, greater infarct volumes following cerebral ischemia, and enhanced kidney and retinal damage. Regenerative processes such as axonal growth and bone formation are also impaired. Although complete PACAP deficiency has not been reported in humans, partial deficiency or low PACAP levels have been documented in diseases such as PTSD and Alzheimer's disease. Therefore, the study of partial PACAP deficiency is especially important. Previous research has shown that partial deficiency is associated with increased oxidative stress and reduced antioxidant capacity. Additionally, older PACAP-HZ mice exhibit symptoms of dry eye and increased retinal cell death following injury. In our current study, we found that the extent of presenile systemic amyloidosis in PACAP-HZ mice was comparable to that observed in PACAP KO mice, reinforcing PACAP's role in protecting against age-related degenerative conditions. These findings highlight PACAP as a potential target for developing future therapeutic strategies for age-associated diseases.

In blood tests, we found no significant differences in complete blood counts between PACAP KO and WT mice, though serum lipid parameters showed slight elevation. These findings suggest that PACAP plays a role in lipid metabolism, consistent with prior studies.

Notably, PACAP KO mice exhibited significantly increased serum creatinine levels, indicating potential renal impairment due to glomerular dysfunction from amyloid plaque accumulation. In PACAP-HZ mice, we observed significant increases in lipid profile parameters, particularly LDL, HDL, and total cholesterol levels. Minor but significant differences were also observed in erythropoiesis and thrombopoiesis, suggesting that PACAP may influence hematopoiesis in addition to lipid regulation.

In our second experiment, we further investigated the effects of aging and PACAP deficiency. Declining PACAP levels with age contribute to systemic alterations affecting nearly all organ systems. Importantly, neuropeptides like PACAP, via the gut-brain axis, can influence immune and stress responses. Elevated PACAP levels have been associated with inflammatory bowel diseases, while its deficiency may increase susceptibility to colitis and colorectal cancer, partly due to reduced beneficial microbiota. Aging diminishes the functional capacity of Peyer's patches, impacting oral tolerance. Our findings confirmed that GALT components like Peyer's patches undergo age-related changes.

Consistent with previous reports, the size of mouse Peyer's patches ranged between 2–4 mm. PP numbers peak during adolescence, and were higher in young (3-month-old) WT mice compared to aging (12–15 months) WT mice. This age-related decline was not observed in PACAP KO mice. Histological analysis did not reveal microscopic differences between young WT and PACAP KO mice. However, significant changes in immune cell populations and immune checkpoint molecule expression were observed in aging PACAP KO mice compared to WT controls, indicating altered immune regulation due to PACAP deficiency.

Flow cytometry confirmed that aging in WT mice was associated with a significant decline in $CD8^+$ T cells, similar to trends seen in elderly humans. Aging $CD8^+$ T cells showed higher TIM-3 expression, reduced Galectin-9 and CD69 expression, suggesting diminished activation. Despite stable perforin levels, granzyme B expression was elevated in aging $CD8^+$ T cells. In contrast, aging WT mice had higher $CD4^+$ T cell frequencies and progressive expansion of CD28-negative $CD4^+$ subsets. These cells showed increased TIM-3 and reduced Gal-9 expression, consistent with regulatory functions. PD-1 expression in aging WT mice aligned with the observed reduction in T cell functionality.

Aging PACAP KO mice exhibited reduced expression of PD-1, PD-L1, Gal-9, and CD69 on $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells, along with significantly higher TIM-3 expression, mirroring results seen in aging WT mice. Functionally, granzyme B expression in $CD8^+$ T cells was increased in aging PACAP KO mice. Contrary to assumptions, the reduced

cytotoxic T cell response seen in aging may not stem from lower cytotoxic potential but rather from impaired clonal expansion of $CD8^+$ T cells.

When comparing young WT and PACAP KO mice, no major differences were found in the frequency of general immune cell populations. However, the $CD4^+/CD8^+$ T cell population in young PACAP KO mice exhibited significantly reduced PD-1 expression and signs of decreased activation, despite increased perforin expression in both $CD3^+$ and $CD8^+$ T cells. These findings suggest that altered checkpoint molecule expression does not automatically result in increased T cell activation.

Aging PACAP KO mice displayed a significant reduction in $CD4^+$ T cells compared to WT mice, accompanied by decreased PD-1 and TIM-3 expression. These results suggest that mechanisms of immune tolerance are altered in the absence of PACAP. However, $CD4^+$ T cell function remained similar to that in aging WT mice. $CD8^+$ T cells in aging PACAP KO mice showed significantly reduced TIM-3 expression compared to aging WT mice. The $CD4^+/CD8^+$ T cell population in aging PACAP KO mice demonstrated increased activity, correlating with decreased TIM-3 expression. This may indicate a compensatory mechanism aiming to sustain immune reactivity despite PACAP deficiency. Despite reduced PD-1 and TIM-3 expression in the $CD3^+$ T cell population of aging PACAP KO mice, granzyme B expression was also significantly lower, underscoring the complex impact of PACAP deficiency on immune regulation during aging.

PACAP plays a fundamental role in maintaining immune homeostasis within GALT by regulating immune cell function and checkpoint molecule expression. Its absence leads to dysregulation, increasing the risk of inflammatory bowel disease and cancer, particularly in older age. The immune profile changes observed in PACAP KO mice reinforce PACAP's protective role in maintaining mucosal immunity.

Through our experiments, we contributed to a better understanding of the pathomechanisms underlying age-associated systemic senile amyloidosis in the absence of PACAP. Furthermore, our findings demonstrate that PACAP deficiency and aging jointly affect the distribution and activation of immune cells within Peyer's patches. These insights may prove valuable in understanding the pathomechanism of IBDs and support PACAP as a potential therapeutic target in managing age-related immune decline and inflammatory bowel diseases.

Summary of Novel Findings - Theses

1. Partial deficiency of PACAP can lead to presenile systemic amyloidosis, in contrast to PAC1 receptor deficiency, which did not induce such pathological changes.

2. In addition to previously described inflammatory and degenerative mechanisms, other factors such as disturbances in lipid metabolism and altered bone marrow activity may also contribute to the development of systemic degenerative processes.

3. PACAP plays a crucial role in maintaining immune homeostasis within gut-associated lymphoid tissue; its deficiency leads to significant alterations in immune cell populations, particularly in aging.

4. Our study simultaneously investigated Peyer's patches at the macroscopic, microscopic, and functional levels, thereby providing a comprehensive insight into the immunological alterations associated with PACAP deficiency in the GALT.

5. PACAP deficiency accelerates the aging process due to the absence of its anti-inflammatory, antioxidant, anti-apoptotic, and cytoprotective effects.

6. Our findings reinforce the pivotal role of PACAP in maintaining immune system integrity and preventing age-associated pathologies.

7. Immunological changes associated with PACAP deficiency may contribute to the development of inflammatory bowel diseases, emphasizing the neuropeptide's role in regulating intestinal immunity.

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List of Publications

1)

Sparks J, Jungling A, Kiss G, Hiripi L, Pham D, Tamas A, Hoffmann O, Bardosi S, Miseta A, Reglodi D.

Presence of systemic amyloidosis in mice with partial deficiency in pituitary adenylate cyclase-activating polypeptide (PACAP) in aging.

Appl. Sci. 2021, 11, 7373.

Q2, Impact Faktor: 2.838

2)

Sparks J, Meggyes M, Makszin L, Jehn V, Lugosi H, Reglodi D, Szereday L.

Effects of PACAP deficiency on immune dysfunction and Peyer's patch integrity in adult mice.

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Q1, Impact Faktor: 4.9

3) 10% of the article's content has been incorporated into the present doctoral dissertation:

Reglodi D, Jungling A, Longuespée R, Kriegsmann J, Casadonte R, Kriegsmann M, Juhasz T, Bardosi S, Tamas A, Fulop BD, Kovacs K, Nagy Z, **Sparks J**, Miseta A, Mazzucchelli G, Hashimoto H, Bardosi A.

Accelerated pre-senile systemic amyloidosis in PACAP knockout mice - a protective role of PACAP in age-related degenerative processes.

J Pathol. 2018 Aug; 245(4): 478-490.

Q1, Impact Faktor: 5.942

The cumulative impact factor of the publications forming the basis of this dissertation is: 13.68