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Cutibacterium acnes: A Proposal for Veterinary Medicine

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Abstract:- *Cutibacterium acnes* (*C. acnes*) is, by far, a highlighted immunomodulator that in recent years has gained prominence for its performance in experimental clinical studies, prophylaxis, and therapeutics of infectious diseases and neoplasms.

If, on the one hand, it can act as an opportunistic pathogen, on the other, can be used as an immunomodulator and in the oncological area. Furthermore, it is also considered a proposal to strengthen the immune system to combat severe acute respiratory syndrome caused by coronavirus 2 (Sars-Cov-2). In other words, its application extends from bacteria, fungi, and parasitic protozoa to viruses. However, even with its growing applications in human medicine, little has been reported in the field of veterinary medicine.

With that said, this review has the purpose of directing the scientific community to the potential of *C. acnes* aimed attreating pathology, especially in animals.

Keywords:- *Cutibacterium Acnes, Immunotherapy, Animals, Veterinary Medicine.*

I. INTRODUCTION

In 1974, the synonymy between *Corynebacterium parvum* (*C. parvum*) and *Propionibacterium acnes* (*P. acnes*) was established [1]. Later, in 2016 [2], with comparative studies of DNA homology of bacterial genera, *P. acnes* was renamed *Cuticubacterium acnes* [3], to differentiate it from other species of the genus *Propionibacterium* [4]. Before the widespread use of *C. acnes*, *Bacillus Calmette Guerin* (BCG) was used as an immunotherapy to treat neoplasms, but due to the systemic risks of using BCG, researchers found in *C. acnes* a safer alternative without systemic risks but with great similarity [5- 7].

Considering its peculiarities, *C. acnes* is a commensal bacterium, facultative anaerobic [8] whose final fermentation product includes propionic acid. Being gram-positive, it is present in the human skin microbiota (sebaceous follicle) [9-11] and can trigger some pathologies, such as inflammatory dermatoses, when that goes out of balance [12-15]. It can modulate various functions of the innate and adaptive immune response, indulging the reduction of systemic infections from multiple origins such as viral 16, bacterial, fungal, or protozoan [17,18], by preventing the evolution of the virulence process or the infective process. Owing to its role in promoting immune defenses, it is currently suggested in the treatment of acute respiratory syndrome, for cases of Covid-19 [19], as a stimulant of the immune mechanism [20], immunomodulator [21], treatment of warts [22], treatment of neoplasms [23], among other pathologies. Besides these encouraging results, there are still punctual studies, not so comprehensive, on the relevance of the use of *C. acnes* for therapeutic purposes used in veterinary medicine, one of the primaries focuses addressed in this review.

II. CUTIBACTERIUM ACNES

Experimental studies in animals

Few scientific papers are available for consultation when it comes to therapeutic actions using *C. acnes* applied to animals. A search on the *Science Direct* platform (https://www.sciencedirect.com/) showed the number of publications on *C. acnes* aimed at its application in veterinary medicine, using the keywords: *Corynebacterium parvum*, *Propionibacterium acnes* and *Cutibacterium acnes* in the period between 1997 and the first half of 2021 (*May)*, (**Figure 1**).

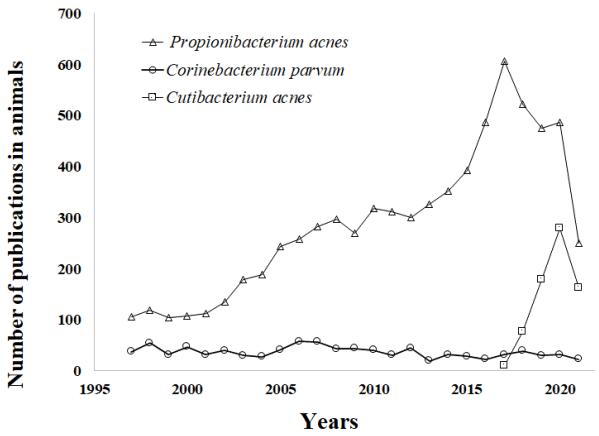

Fig. 1. Evaluation of scientific publications presented by *Science Direct* for publications with *C. acnes* applied to animals, (a search carried out in May/2021).

The immunogenic potential using *C. acnes* lysate started to be investigated in clinical studies in rats [24], showing a relationship between the activation of the immune system and the stabilization, regression or increase in the longevity of animals transplanted with a high-rate tumor cells mortality. This was confirmed by Andre; Zitvogel; Sabourin, [25], where the authors studied the performance of two vaccines

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(*Wellcome Reagents*, Ltd. and *Institut Merieux* - Lyon, France), containing a suspension of inactivated *C. acnes*, to indulge immune reactivity in mice with breast carcinoma. The animals were treated for a period of 3 to 12 days with subcutaneous applications of bacterial lysate, by intravenous, intraperitoneal and intratumoral administrations. Tumor regression and inactivation were observed. The authors report that intratumoral injection thirteen days before surgical removal prolongs the survival or cure of treated animals.

Braga and collaborators [26], treated subcutaneoussly (murine model) mice sensitized by ovalbumin, inducing so an allergic pulmonary response. Therapy followed the application of 700 mg of *C. acnes*, resuspended in 350 ml of saline, once a week (subcutaneously), for a period of three weeks. In the second week, the animals were treated for seven days with a suspension of *C. acnes*, and in the last week, the animals were sensitized with ovalbumin. It was observed that *C. acnes* acted to modulate the late phase-type I hypersensitivity reaction. It is reported that subcutaneous injections of *C. acnes* lysate over three weeks induced specific antibody responses, and increased the phagocytic capacity of peritoneal macrophages. In a period, shorter than 24 h, after a single intraperitoneal inoculation, there was a significant increase in macrophages, immature dendritic cells, and Natural Killer (NK) cell population. Prompt treatment before sensitization modulated the specific immune response, promoting a Th1 response, preventing hypersensitivity to the allergen.

Abreu and collaborators [27], presented the case study where dogs affected with oral papillomatosis were treated with an association between Vincristine sulfate and suspension of *C. acnes*. Also considering the lack of an effective treatment for this purpose, the authors used *C. acnes* as an immunostimulant, together with liposaccharides from *Escherichia coli*. The therapeutic protocol was performed by weekly applications of Vincristine, intravenously, starting with the first dose of 0.025 mg/kg; in the second week, 0.035 mg/kg and thereafter, 0.05 mg/kg. 1.0 ml of the suspension of *C. acnes* was applied intramuscularly (1.0 ml per kg) every 15 days. It was observed that from the third week onwards there was regression of the papillomas followed by healing after three months. Due to the success of the therapy, the authors suggest that this protocol becomes an alternative for cases resistant to the usual therapy to *Thuya occidentalis*.

Manickam and collaborators [28], used inactivated *C. acnes*, as an adjuvant immunizing agent in a group of calves for production of non-specific resistance to protozoan *Theileria annulata* present in ticks. Crossbred calves (*Bos indicux* × *Bos taurus*) received 200 mg of *C. acnes* reconstituted in 2.5 ml of distilled water subcutaneously. After a period of 45, 60 or 90 days past immunization, the calves were challenged with ticks infected with *Theileria annulata*, where only mild reactions were observed. All other animals in the control group that did not receive the bacterial lysate died due to *Teileriosis* [28].

On the other hand, the authors Dinsmore and collaborators [29], reported low efficiency of *C. acnes* for dairy cows affected by *Staphylococcus aureus*, which causes infection of the cows mammary quarters. Milk obtained from milking was analyzed for a group of 51 lactating cows. Twenty-four cows received 1.0 ml injections of the *C. acne* suspension, twice a week, for a period of one month, while the others did not receive the bacterial suspension. 16.7% of treated cows were cured of *S. aureus* and 11.1% of untreated cows were cured, regardless of the use of bacterial lysate. Therefore, showing a not-so-significant effect of the immunomodulator. The authors, however, do not report the dosage applied in terms of bacterial lysate mass. The application and efficiency of *Cutibacterium acnes* were investigated experimentally in animals and are shown in **Table 1**.

Case Study

In the process of a bibliographic survey for the construction of this review proposal, few presentations regarding "Case Studies" are available for consultation in the field of veterinary medicine. However, an interesting study presented by Abreu and collaborators [27], successfully investigated the use of *C. acnes*, as an adjuvant in the treatment of *Papillomatosis*, as images are shown in **Figure 2**. The authors' used therapy consisted of the use of Vincristine associated with an immunostimulant based on *C. acnes* lysate and *Escherichia coli* liposaccharides. The bacterial lysate was administered intramuscularly every 15 days, at a dosage of 1.0 ml (1.0 ml/10 kg), for a period of six months, after intravenous administration of Vincristine sulfate at a dosage of 0.025 mg/kg in the first week; 0.035 mg/kg in the second week; and subsequently 0.05 mg/kg. A full recovery of the animals was reported.

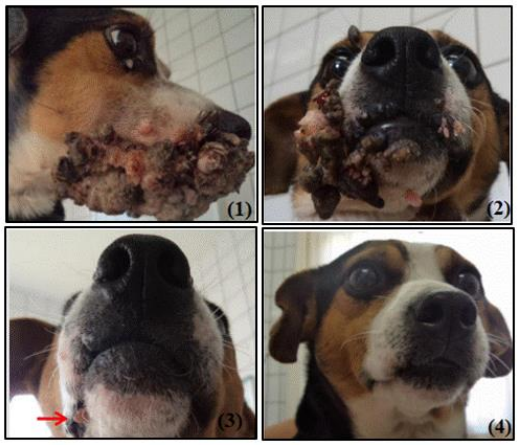

Fig. 2. Results obtained by Abreu and collaborators [27], using *C. acnes* as an adjuvant in the treatment of canine oral *papillomatosis*. (1-4) Progressive evolution over 6 months with 15/15 days of administration of *C. acnes* with adjuvant/ Vincristine.

Table 1. Experimental studies using *Cutibacterium acnes* in animals.

Therapy	Application
Adjuvant/ Immunostimulants	Primary tumors (mouses) [30]
Nano-immunotherapy	Melanoma (mouses) [31]
Nano-immunotherapy	Murine hepatitis (mices) [32]
Immunotherapy	Oral melanoma (dogs) [33]
Immunotherapy	Dermatitis/Pyoderma (dogs) [34]
Immunotherapy	Tumor (mices) [35]
Application under bone marrow-derived dendritic cells	Tumor (mices) [36]
Immunomodulator/Protector against infections	Rabies virus infection (mices) [37][38]
Fragmentation of *C. acnes* by nanotechnology	Taxological risk assessment (monkeys) [39]
Anti-inflammatory	Sepsis / Inflammation (mices) [40]
Immunotherapy	Inhibition of tumor (mouses) [41]
Protective effect	Hepatitis (mouses) [42]
Immunomodulator /viral resistance	Infection with Herpesvirus hominis (mices) [43]
Adjuvant with vaccine	*Fungo Paracoccidioidomycosis* (mouses) [44]
Adjuvant with vaccine	*Leishmania major* [45]
Immunotherapy	Malignant melanoma (dogs) [46]
Immunotherapeutic agent	Inhibition of *Sarcoma* [47]
Antitumor, Anti-Angiogenesis and Immunomodulatory Effects	Breast cancer (mices) [48]
Anti-tumor/Immunological adjuvant	Tumor (mices) [49]
Stimulant of the reticuloendothelial system	Burn treatment (mices) [50]
Infection prophylactic	Pleuropneumonia (porcines) [51]
Adjuvant/ Immunomodulator	Inflation reduction (mices) [52]
Pre-treatment / antitumor	Solid tumor reduction (mices) [53]
Immunomodulator	Brucellosis (guinea pigs) [54]
Immunomodulator	Papillomatosis (dogs) [55][56]

III. ACTION MECHANISM IN TUMORS

Over decades, studies with *C. acnes* have shown its antitumor action, demonstrating the inhibitory or regressive activity, under conditions of neoplasms, in animals and humans [57-63], but the exact mechanism of action of *C. acnes* in inducing tumor growth delay or regression is still unknown. However, its immunomodulatory effects are reported, mainly due to its ability to activate cellular functions of the mononuclear phagocytic system [64-65]. The stimulation of the reticuloendothelial system has been repeatedly confirmed [58,66], and shows the increase in the non-specific immune response and the activation and multiplication of macrophages and regulation of the immune response of NK cells and T lymphocytes [67-69].

Immune Response

When *C. acnes* lysate is injected into the body, the immune system "perceives" the presence of these particles, initially, through phagocytic activity and possible cell types: macrophages, neutrophils, dendritic cells, leukocytes (NK), mast cells, basophils, eosinophils or keratocytes [70]. *C. acnes* simulates a pathogen that crosses the skin barrier, which activates innate immunity, using one of the cell types mentioned above. After phagocytosis, PPR (Pattern Recognition Receptors), TLR (Toll-Like), NOD (Domain Nucleotide Oligomerization), RIG-I (Retinoic Acid Inducible Gene) and C-Type Lecithin receptors are expressed [71]. PPR recognizes different pathogens that are associated with the PAMP pattern (Pathogen-Associated Molecular Pattern) or cause cell damage (DAMP) and consequently induce the production of pro-inflammatory substances (cytokines, TNF-alpha and IFN-gamma and chemokines (which recruit more phagocytic cells).

The starting point for immunostimulation from *C. acnes* lysate occurs when NK cells are activated and this is the main proposal for its use. At this moment, a quick response is stimulated to the possible bacterial, fungal or protozoal infection, and if it is fast enough, it will prevent the invading organism from reaching other tissues. When infection occurs by a virus, the initial immune reaction is triggered and activates the adaptive system as follows: When NK cells phagocytose the *C. acne* lysate, they maximize the production of initial INF'a increasing the antiviral status [72] which, concomitantly, stimulates promyelocytic leukemia nuclear bodies to associate with histone chaperones to capture the viral genome and block its replication [73-74]. Keratocytes also release IL-1 alpha and IL-36 to enhance the antiviral state, acting as early warning signals for leukocyte chemotaxis and increased cellular sensitivity to type I IFN signaling [75]. In practice, both an innate and an adaptive response are initiated concurrently, regardless of the type of invading organism.

Side and Adverse Effects

The use of *C. acnes* lysate in most experimental studies shows few or rare adverse reactions, thereby, its use is well tolerated. Among the reported side effects there are fever and chills [76]. Fever is an expected side effect as substantial amounts of liposaccharides are being injected and the chills are an effect of the fever. However, some authors have observed adverse reactions in mice treated with the bacterial lysate, such as the development of intrahepatic granulomas and scleromas in injected areas [77].

The formation of granulomas and scleromas can be related to applications of exceedingly high concentrations in the same place, which causes an intense inflammatory process in the region of application. It should be noted, however, the dosage to be applied in each specific case, especially regarding its

combination with other substances, such as cyclophosphamide, for example [78]. In an attempt to minimize possible adverse reactions, studies in the field of nanotechnology have been developed based on the encapsulation of bacterial lysate, with the advantage of being easily absorbed and released in low concentrations in the body [31].

Clinical Application

C. acne lysates were used in peritoneal applications [79], intravenously or directly infiltrated into the neoplastic nucleus, in animals [80] and humans [60]. As for their use on a global scale, *C. acnes* lysates have never been industrially traded for human use outside Brazil, where the LAFEPE laboratory (*Pharmaceutical Laboratory of Pernambuco*) had the registration of *C. acnes* lysate with the trade name of IMUNOPARVUM®, its orientation for use is 5 deep subcutaneous applications with volumes from 0.5 ml to 1.0 ml for 7/7 days. After this period, an assessment of cellular immunity with PPD (Tuberculin or Mantoux reaction), Candidine, or Tricophytin tests must be performed, if it reaches normal levels of cutaneous reactivity. The sequence of applications must be carried out for 15/15 days, being the duration defined by medical criteria according to the package insert. Another product, in this case only for veterinary use, INFERVAC® has also been marketed. In this case, indicated doses were 2.0 ml for animals weighing up to 10 kg and at intervals of 48 hours, the concentration of *C. acnes* doses reaches 400 mg / ml [81], it is no longer marketed.

Currently (2021), in Brazil, only the *Laboratory of Allergenic Extracts* LTDA, holds the registration of the *C. acnes* lysate, with the name PARVULAN® at the concentration 2.0 mg/ml in 5.0 ml vials and application guidance of 0.5 ml for 7/7 days (ten doses). In the country, most of the *C. acnes* lysate is marketed in a manipulated way and used as an adjuvant in immunotherapy formulations. A major difference between the use of bacterial lysates from the upper respiratory tract and *C. acnes* lysate is that the latter is applied in an injectable way, specifically deep subcutaneously, causing a systemic action, contrary to bacterial lysates.

IV. CONCLUDING REMARKS

The *C. acnes* lysate shows immense potential in different applications, from solving an immunological problem of "low immunity", improving the body's defense capacity against bacteria, fungi, viruses and protozoa, to an effective application in different grades of neoplasms. Its activity seems to be dose and interval-dependent, behaving as an immunomodulator at low doses and long application intervals (interesting use to control chronic inflammatory processes) and as an immunostimulator with high doses and short intervals. This allows for appropriate management for different pathologies and can even be used as an adjuvant, enhancing the synergism of vaccines and many medications (antibiotics, antivirals). Its use in neoplasms is better in closer or intratumoral doses, that is, it is limited to neoplasms that are within reach of the syringe needle.

Application in veterinary medicine is very promising, as it is much less invasive than surgery and does not leave scars, and reduces the chances of metastases, in addition to the fact that surgical anesthetics often work as immunosuppressants, which is bad for recovery, it can still be monthly, quarterly or biannual doses with prophylactic function.

Conflicts of interest

There are no conflicts of interest associated with the publication of this article.

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