CORYNEBACTERIUM PARVUM

Applications in Experimental and Clinical Oncology

Edited by

Bernard Halpern

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Preface

In his introduction to the Ciba Foundation Symposium on "Immunopotentiation," Sir Peter Medawar stated that, "for the past twenty years the control of the immune response has been virtually equated to immunosuppression because the great goal of applied immunology has been the transplantation of tissues," and that, "with the discovery of tumor immunity–the focal point of immunological control has changed from immunosuppression to immunpotentiation and correspondingly the great prize of applied immunology has become the prevention and control of malignant growth." *Corynebacterium parvum* is now universally recognized as being the most potent presently known immunopotentiator.

C. parvum is at the center of almost all papers presented at this conference and it has been analyzed from various viewpoints: taxonomy, pharmacology, immunopotentiation, stimulation of host resistance to tumor invasion in experimental models and in human therapeutics. Almost all data reported in this volume are original and recently obtained. The discussions were spontaneous, stimulating, and highly enriching, and the results already established and reported here are in many respects unique. Discussions at the conference revealed many other aspects which require further exploration.

Nonspecific immunopotentiation has strongly penetrated into immunology, immunopathology, and, recently, oncology. Undoubtedly, this area of research will witness important developments in the near future.

This book offers the first synthesis of presently available knowledge and emphasizes future prospects.

These proceedings should interest immunologists working in basic and applied immunology, as well as those concerned with the mechanism of tumor immunity and cancer immunotherapy.

Paris, June 1975

Bernard Halpern

Opening Address

It is a great pleasure for me to welcome you to the College de France, one of the oldest and most universal schools of thought and science throughout the world. May I remind you that the College de France was founded in 1531 by King François I, at a moment when the western world was emerging from the Middle Ages and when the breath of the Renaissance was illuminating civilization. The ambitious motto of the College de France is *Docet omnia*, and the institution has not failed to live up to this device so far.

This meeting, devoted to the study of the effects of *Corynebacterium parvum* in experimental and clinical oncology, comes at the right time. It so happens that this year is the tenth anniversary of the first paper published Halpern *et al.*, 1964 on the strong stimulatory effects of *C. parvum* on the reticuloendothelial system. Since then, much information has been reported from different laboratories concerning the immunopotentiating properties of this bacterium, such as potentiation of antibody synthesis, stimulation of cell-mediated immunity, and antibacterial and antiviral activities.

There is also now accumulating evidence that *C. parvum* affects neoplastic growths and inhibits metastatic dissemination. Although this evidence originates mainly from experimental data, the flow of clinical data corroborates the experimental findings to a great extent. However, *C. parvum* is of a highly complex nature, and the mechanisms which are instrumental in its antitumor activity are only hypothetical at the present time. It will be the aim of this meeting to analyze this essential problem in the light of available data, and to suggest new experimental models that are likely to illuminate this field.

This meeting brings together bacteriologists, biochemists, pharmacologists, biologists, oncologists, and clinicians. It may be hoped that the data presented will be of mutual interest and that the discussions which will follow will suggest new avenues of investigation.

BERNARD HALPERN, Professor of Experimental Medicine, College de France, Paris (France).

Recent advances in immunology have stressed the complexity of immunocompetent-cell interactions. The results which will be reported here must find their explanation in the light of these fundamental facts. While *C. parvum* is known to activate macrophages, and probably also B lymphocytes, the effect on T lymphocytes is less evident.

Another important aspect which should be discussed is the real importance of the so-called enhancing antibodies in the antitumor effects of *C. parvum*. Finally, this conference should lay down some principles for clinical applications and criteria on which the appreciation of clinical results should be based.

Nonspecific immunopotentiation is a new breakthrough in fundamental and applied immunology, particularly in oncology. The ground rules of this approach have still to be established. It will be the goal of this conference to accomplish this.

Acknowledgments

I wish to express my very great thanks to Professor Etienne Wolff, President of the College de France, who kindly put at my disposal for the purpose of this conference the lecture room, the personnel, the cafeteria, and many other facilities which made this conference successful.

I am also addressing, in your name as well as my own, sentiments of gratitude to Dr. Charles Mérieux, Director of the Institut Mérieux, and to his deputy director, Dr. R. Triau, whose generous financial support made this conference possible. Dr. Mérieux is a true Maecenas in the biomedical sciences, and countless are the conferences and research programs which he has subsidized so generously. His sentiments of personal friendship and kindness toward me are deeply appreciated.

I would like to express my best thanks to my secretaries, Mrs. Elisabeth Launay, Mrs. Betty Sossler, and Miss Ghislaine d'Hérouville, for their devoted and untiring help in the organization of this conference and their assistance in my editorial endeavors. I must also congratulate Palantype, London, for the splendid job they did in transcribing the recorded tapes.

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Part I
Bacteriology and Taxonomy

1

Bacteriological Aspect of Anaerobic Corynebacteria in Relation to RES Stimulation

A. R. PRÉVOT

Background

Anaerobic corynebacteria have been known since 1897, when Roux first described *Corynebacterium pyogenes*. Roux, a veterinary doctor practicing in the Ile-de-France, observed an enzootic disease of purulent abscesses affecting cattle (hence its incorrect name of *C. pyogenes bovis*). Roux isolated the germ which caused the disease and studied it at the Pasteur Institute. In 1908 and 1909, in Metchnikoff's laboratory at the Pasteur Institute, Jungano was studying fecal microflora and discovered three species of corynebacteria: *Corynebacterium liquefaciens*, *Corynebacterium diphtheroides*, and *Corynebacterium granulosum*.

The first notion of their pathogenicity was revealed in 1913 by Massini, who isolated several strains of *Corynebacterium anaerobium* from cases of septicemia, mastoiditis, and purulent adenitis. *C. anaerobium* later proved to be the most frequent and most pathogenic of the anaerobic corynebacteria. As the type of this subspecies, we chose *C. anaerobium* for our experiments.

It was Torrey who, in 1916, first stressed the reciprocal affinity between

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anaerobic corynebacteria and the RES when he described the *Corynebacterium lymphophilum* species.

In 1926, Mayer described *Corynebacterium parvum*. In the work carried out in my laboratory, *C. parvum* was the first species to reveal the secret of its pathogenic mechanism and, therefore, its capacity to stimulate the RES. Later on, other, less important species were described: *Corynebacterium hepatodystrophicans* (Kuczinski, 1929), *Corynebacterium renale cuniculi* (Manteufel and Herzberg, 1930), and *Corynebacterium avidum* (Eggert, 1935).

Personal Research

It was in 1938 that I undertook my research on this interesting group of bacteria. I began by publishing a first attempt at classification of the species already described, taking as the basis for their taxonomy the morphological and physiological characteristics briefly outlined by the various authors (Prévot 1938; 1946). That classification attempt showed that the various species of corynebacteria clearly differ in their enzyme patterns. Since at that time, the still existent axiom, "1 enzyme = 1 gene," had already been recognized, it may be hoped that they are genotypical rather than phenotypical species. The classification enabled me gradually to account for all of the species, to complete their description, and to study their pathogenicity, which was so unexpected, and, consequently, their capacity to stimulate the RES (Prévot *et al.*, 1949; Prévot, 1960). One of the observations which impressed me most and was to lead me to study in depth the relationship among these anaerobes and certain malignant RES diseases dates back to 1949. With Courdurier (Prévot *et al.*, 1949), I isolated by anaerobic hemoculture a strain of *C. avidum* in an elderly woman suffering from episodes of recurrent septicemia, lasting seven days, from whom it had been impossible to isolate any of the anaerobic organisms normally looked for in such cases. For several months, this recurrent septicemia resisted all treatments, leading to the death of the patient. The postmortem revealed a malignant lymphogranulomatosis, the characteristic symptoms of which had never appeared. At a later stage, this coincidence enabled us to find a relationship between the anaerobic corynebacterial septicemia and certain malignant RES diseases, which (in our statistics) accounted for as many as 11% of the cases.

We observed a second case of septicemia caused by *C. avidum* (Prévot and Huet, 1951) and published a study (Prévot and Tardieux, 1953) on the pathogenicity of anaerobic corynebacteria. In 1960, I was able to present a comprehensive study of these infections (Prévot, 1960), in which endocarditis played an important part (Prévot *et al.*, 1954a; Prévot, 1956; Prévot *et al.*, 1956a). Our coworker, Mme. Mandin (1956), reported approximately thirty cases (observed in the clinic of Prof. Janbon at Montpellier) in which recurrent septicemia led to a malignant RES disease.

The study of the culture characteristics of our 600 strains of anaerobic corynebacteria showed some peculiarities of this group and, above all, their great variability. We will stress here only two such characteristics: first, they can be isolated only under strict anaerobiosis. Second, the very first colonies isolated are exempt from catalase; however, in the second subculture, a catalase appears, and the strict anaerobiosis is no longer necessary.* In deep gelose, the levels of the colonies rise progressively, first appearing at a depth of more than 1 cm below the surface and rising to within a few millimeters from the surface. Some strains may even give facultative anaerobic mutants but, on the whole, they remain preferential anaerobes (Prévot and Thouvenot, 1952). Another aspect of their variability is that most strains, even when isolated in a pure state from serious microbial septicemia, lose all experimental pathogenicity as early as the first culture. This led several authors who had studied only a few strains—which were precisely such apathogenic mutants—wrongly to deny the pathogenicity of anaerobic corynebacteria. Other strains which retain this pathogenicity during the first cultures gradually lose it with successive subcultures. It is probably because of this variability that the understanding of their unexpected pathogenicity was delayed for so long.

Study of Pathogenicity

This study could be carried out only when we were able to use strains which were pathogenic to animals and remained so. The study was undertaken with Dezest and, in particular, with Levaditi and his team (Levaditi *et al.*, 1965; Prévot *et al.*, 1954b; 1955; 1958), and also with other authors. When a guinea pig is injected intramuscularly or intravenously with 1 ml of a 24-h culture of a highly pathogenic strain, the animal dies either rapidly within 24–48 h, exhibiting local infectious reactions and purulent metastasis (purulent adenitis, pleurisy, peritonitis, etc.), or within 10–12 days. In the latter case, there are no massive reactions, and only the histopathological examination of the lungs, liver, spleen, and kidneys reveals an acute histioreticulosis with large multinuclear macrophages showing intense hyperergia. In the former case, anaerobic corynebacteria are easily isolated from the lesions, but this is very difficult and sometimes impossible in the latter case where the reactions are, in fact, due to self-sterilizing experimental infections. In both cases, we then observed—and this is pathognomonic—a hypercytosis in the four clones of the RES: reticulated Aschoff cells,† lymphocytes, reticulocytes, histiocytes, and macrophages. Thus, the injection of living anaerobic corynebacteria induces a lethal stimulation of the RES. However,

*Some authors thought they could assert the existence of a catalase in the first culture after isolation. It seems that the technique they used was not sufficiently discriminating to detect the absence of initial catalase.

†At that time these cells were called reticulocytes. This word now has another meaning in hematology: reticulated red cells.

when we tried with Linzenmeyer (1954), to obtain sera to agglutinate these corynebacteria by intravenously injecting cells killed by heat or formaldehyde, we found that the high doses required for the 9th, 10th, and 11th injections killed the rabbits and that the histological examination revealed the same epithelial hyperergia, the same lethal stimulation of the RES, and the same hypercytosis of the four clones of RES.

Therefore, it is not the virulence of the anaerobic corynebacteria that is at the basis of their pathogenicity, but the action of one of the components of their cells, whether living or killed. We tried to determine with Tam (Prévot *et al.*, 1968) which substance was responsible for the pathogenicity. We identified it as being one of the constituents of the bacterial cell wall. This was later confirmed while working with another team (Prévot *et al.*, 1972), and we initially suggested calling the pathogenic component reticulostimulin (Prévot, 1965a) to express its potential to stimulate the RES.

When we successfully titrated this substance (Prévot *et al.*, 1963; Prévot, 1964) by using the Halpern and Biozzi method, our research changed from qualitative and became quantitative. We were then able to define the pathogenic substance as being "a parietal constituent of pathogenic anaerobic corynebacteria which, at high doses, is capable of causing a lethal hyperergical histioreticulosis and, at the therapeutical doses, brings about a beneficial stimulation of the RES, involving a remarkable increase of the natural defenses of the system."

Before describing the research which followed this turning point in our investigations, it should be noted that one of the most serious anaerobic corynebacterioses is Whipple's disease, which we showed to be an infection of the lamina propria by *C. anaerobium* (Caroli *et al.*, 1963; Prévot and Morel, 1964). This disease always reveals itself to be a fatal mesenteric histioreticulosis; when treated with specific antibiotics, in particular chloramphenicol, this infection can now be cured (Prévot, 1965b). It has two characteristics: It is self-sterilizing and, at that stage, not only is it impossible to isolate the organism, but a large number of intestinal bacterial species are expelled. This is the reason why many authors dispute the corynebacterial origin of infection, having only studied the disease after self-sterilization (Prévot, 1965b) and after associated reinfection.

With regard to recurrent septicemia ending—sometimes after a very long delay—in a malignant RES disease, we formulated the assumption that these recurrences finally blocked the RES after repeated stimulations, causing the system to become the prey of unknown agents of malignancy (oncogenic viruses in particular) (Prévot, 1972).

Research on Reticulostimulin

The titration of reticulostimulin (RS) by the colloidal carbon method enabled us to carry out the following research: