NETWORK OF CENTRES OF EXCELLENCE PROGRAM

Canadian Bacterial Diseases Network: a new approach to university-industry relationships

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Abstract

Bacterial diseases are a substantial problem worldwide. Their diagnosis and therapy form the basis of a multibillion dollar industry. This industry is dynamic and is continuously revitalized by research. The Canadian Bacterial Diseases Network (CBDN) was established to capitalize on the enhanced opportunities that now exist for the rapid progression of an idea from conceptualization to implementation and, ultimately, commercialization. CBDN is one of 15 Networks of Centres of Excellence, a Federal Government initiative whose intention is to improve Canada's economic competitiveness in the global market. CBDN research involves fundamental science, is broadly-based, and encompasses all aspects of bacterial diseases. Current projects include the investigations of strategies to block *Pseudomonas aeruginosa* binding to epithelial cells and a novel anti-toxin approach for *Escherichia coli*. Also CBDN is investigating the basis for antibiotic resistance in Gram-negative bacteria, and is devising improved procedures for overcoming such resistance mechanisms. CBDN is only 28 months into its first 4-year mandate; however, considerable successes have been enjoyed to date.

Résumé

Les infections bactériennes sont un problème d'importance mondiale. Leur diagnostic et leur thérapie représentent une industrie de plusieurs billions de dollars. Cette industrie est dynamique et continuellement revitalisée par la recherche. Le Canadian Bacterial Diseases Network a été établi pour profiter des opportunités actuelles permettant un progrès rapide d'une idée, de la conceptualisation à l'implémentation et à la commercialisation. Ce regroupement est un des 15 Centres d'Excellence du Canada, une initiative Fédérale dont l'intention est d'améliorer la compétitivité canadienne sur le marché mondial. Ce réseau de recherche implique les sciences fondamentales et est largement établi, recouvrant tous les aspects des maladies bactériennes. Les projets en cours actuellement se penchent sur la stratégie pour bloquer la liaison *Pseudomonas aeruginosa* épithélial et la production nouvelle d'anti-toxines contre *l'Escherichia coli*. Ce groupement évalue également les fondements de la résistance aux antibiotiques chez les bactéries Gram-négative et prépare de nouvelles stratégies pour faire face à cette résistance. Notre regroupement n'a que 28 mois et a cependant connu jusqu'à présent un succès tout à fait remarquable.

Introduction

In May 1988, the Federal Government and the three granting councils, the Medical Research Council of Canada (MRC), the Natural Sciences and Engineer-

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ing Research Council of Canada (NSERC) and the Social Sciences and Humanities Research Council of Canada (SSHRC), announced a new program for Canada, the Networks of Centres of Excellence (NCE) Program, which was to be funded with new money at the level of $240 million over 5 years. There were 2 lines of thinking underpinning the NCE program. The first was based on the Government's desire to increase Canada's economic competitiveness. Traditional Canadian pursuits as providers of raw materials, fish, wood, wheat, etc., did not seem to be sufficient to maintain the high standard of prosperity enjoyed by Canadians, a prediction borne out by recent events. Thus, the Government was seeking ways to both make our current industries more competitive and to build strong, new industry sectors. Given Canada's high standard of education and reputation for innovation, it seemed reasonable to promote the development of high technology sectors. Thus, the NCE program's first major objective was to build stronger linkages between industry and the university/government research sectors based on mutual respect and an understanding of the needs of both sectors. The second objective was based on the geographic reality of Canada, a country slightly larger than the USA, but with one-tenth the population. Hence, by creating Networks that genuinely linked researchers across Canada, and providing researchers with the funds and mechanisms for continuing interaction, the combined strength of these researchers in a given discipline might be expected to rival that of world-renowned institutes, like the National Institutes of Health in Bethesda and the Scripps Institute in San Diego, or that of highly populated areas like the San Francisco Bay and New York areas.

The program was developed at a staggering rate, with 158 full-sized applications submitted by November 15, 1988, involving some 4000 researchers and $2 billion in requests. These applications were then ranked by an International Committee, the top 50 were reviewed and their sites visited, and decisions were made by July 1989 and announced October 26, 1989. Fourteen successful networks were later joined by a fifteenth, funded by other moneys. These networks spanned many areas of investigation including human health, agriculture, information systems, lasers, and high performance concrete technologies. Six biotechnology networks were funded including: Canadian Bacterial Diseases Network, Canadian Genetic Diseases Network, Protein Engineering Network of Centres of Excellence, Neural Regeneration and Recovery Network, Respiratory Health Network of Centres of Excellence, and Insect Biotech Canada. After negotiation with all parties involved, including the NCE Tri-Councils committee represented by an implementation committee chaired by Stuart Smith, the participating researchers, universities, and government research agencies and companies signed comprehensive operating agreements, termed "Internal Agreements". The networks then came into being between April and September, 1990. CBDN was actually the first of the networks implemented and, from this perspective, is the most mature. However, it is important to note that the lifetime of this network was only 28 months at the time of writing and this must therefore be considered an interim report. However, CBDN believes that they have enjoyed considerable success to date, as have many of the NCE's, and is progressing towards creating a new research environment that will meet the objectives laid out by the Federal Government.

The mission

CBDN has as its mission statement, "To advance scientific knowledge and enhance Canada's economic competitiveness through networking, excellence in fundamental research on bacterial diseases and collaboration with industry (Putting fundamental science to work)". Thus, its identity is revealed by its name as a Canadian group involved in highly interactive (networked) research on bacterial diseases.

CBDN combines the expertise and pooled resources of internationally recognized scientists whose skills encompass the use of sophisticated techniques and approaches such as gene and protein engineering, monoclonal antibodies, scanning and transmission electron microscopy, 2-dimensional nuclear magnetic resonance spectroscopic analysis of polysaccharides, a wide variety of novel bacterial infection models, fluorescence microscopy and spectroscopy, model membrane and liposome reconstitution methodologies, and a broad spectrum of immunological experience and immunochemical techniques. These techniques and the experience of

the investigators involved are being utilized to develop novel approaches to disease-specific and more general problems of bacterial pathogenesis.

The research program of CBDN provides a bridge linking fundamental and applied science. While concentrating on "basic" science, the CBDN research effort is highly orientated toward projects that address the concerns of industry, with the goal of transferring technology to industry to permit development of products. Through CBDN the biotechnology and pharmaceutical industries have the opportunity to gain access to a unique source of expertise and intellectual property in fundamental research on bacterial diseases and related disciplines affecting humans, animals, and plants. The CBDN research program, outlined below, is of direct relevance to the production and enhanced efficacy of antibiotics and vaccines, and to the development of novel approaches to diagnostics.

CBDN comprises established scientists with strong international reputations, younger scientists with excellent credentials, and industrial partners with a sound reputation for research. Included among these scientists are 2 winners of the world's major prize for antimicrobial chemotherapy research, the Hoechst-Roussell Award (Dr L.E. Bryan and Dr J. Davies), a Roy L. Whister International Award winner for carbohydrate chemistry research (Dr D.R. Bundle), a Steacie Award winner (Dr T.J. Beveridge), four Canadian Society of Microbiologists Award winners (Drs L.A. Babiuk, L.E. Bryan, R.E.W. Hancock, and M.B. Perry), a Canadian Infectious Disease Society Award winner (Dr A.W. Chow), a winner of the Diamond Jubilee Award in Agricultural Research (Dr L.A. Babiuk), and a Howard Hughes Fellowship winner (Dr B.B. Finlay).

Uniqueness

We believe that the NCEs are rather unique organizations. Certainly in the biotechnology/pharmaceutical sector there are no national organizations structured like the NCEs, and this is certainly true in the area of bacterial diseases research. Thus, this is truly a made-in-Canada program. Further, a balanced perusal of the information contained within this article should lead the reader to the conclusion that research funded through the NCE program is fundamentally different from that normally funded by the federal granting agencies. A genuine collaborative environment and requirement to interact productively with industry and the constraints provided by the co-signed Internal Agreement all provide limits on intellectual freedom, although there are compensations for these limitations. Moreover, there is a widely held perspective that the money provided to the NCE program was made available to meet specific federal government objectives (see above), and thus is unlikely to be made available to MRC and NSERC if this program fails.

Management

NCEs are large and complex organizations. CBDN, for example, has 37 members (project leaders) at 7 universities, 2 government agencies (NRC and LCDC), and a small biotechnology company. In all there are 226 research personnel employed by CBDN funds, including 125 research trainees (postdoctoral fellows and graduate students). Hence, CBDN has a major involvement in the training of molecular microbiologists in medical biotechnology that is relevant to industry. Counting members, research associates, and postdoctoral fellows on salary, we have 101 PhD microbiologists working on CBDN projects, which is a considerably greater number than most pharmaceutical company giants. Such an organization requires an effective management structure. In fact, CBDN is managed like a small company, although it is not incorporated. The final decision-making rests with an independent Board of Directors (E. Geddes, Chair, W. Cochrane, R. Murray, A. Ronald, R. Sheinin, and G. Stewart). The Board of Directors works through a scientific director, Bob Hancock, and a managing director, Henry Geraedts, who are non-voting members of the Board, and a management committee, the Network Science and Budget Committee. This latter committee, comprising representative members from the 8 cities in which members are located, is responsible for overseeing the research program and budgetary matters, and for making recommendations to the Board of Directors. CBDN has shown decisiveness in management of its research program. In January 1992, a comprehensive internal review of the Network's research activities was undertaken, including site visits to each research

centre by panels which included external experts. As a result, CBDN decided to discontinue 7 projects, and to sever its relationship with 10 members; while an additional 4 projects were completely revamped, 9 new projects were funded and 4 new members appointed. CBDN has recently initiated a process aimed at identifying prominent Canadian researchers and institutions which were not part of the original application but would contribute to the objectives of CBDN. These researchers would then be invited to join this organization. Thus, we feel that CBDN is ensuring that it pursues its mandates of research excellence, industry relevance, and networking.

Research program

CBDN was awarded a sum of $18.2 million over four years. Most of this research money has been directed to personnel and supplies, and relatively little has gone to equipment, since the Network is generally well equipped. For example, CBDN has available at NRC one of the best carbohydrate analysis facilities in the world, an excellent state-of-the-art electron microscopic facility at the University of Guelph, several sophisticated molecular biology laboratories, and access to nearly every other technique of importance to bacterial diseases research. Infrastructure costs to support CBDN research have been provided by the provincial governments and/or the employing centres of members.

CBDN has a very broad-based research program with 39 individual projects (Table 1). Each of these projects has a project leader and one to several research collaborators who are contributing substantially to the research goals (e.g. Fig. 1). In all, 234 collaborative interactions were identified in the *January 1993 Annual Report*. There is insufficient space to describe the entire research program here, so just a few projects will be mentioned.

CBDN's research covers all aspects of bacterial diseases. Since bacterial infections share common features regardless of the host, the Network is performing research on diseases of humans, food animals, fish, and plants using the same basic technologies. A large percentage of the major causative agents of bacterial diseases in humans are under study and, where gaps in CBDNs repertoire exist, it is their intention to fill them by recruitment of new members over the next few years.

Bacterial diseases are a substantial problem worldwide, and their diagnosis and therapy form the basis of a multibillion dollar industry. Although dollar figures are difficult to come by, it has been estimated that antimicrobial drugs represent $4 billion in sales in the USA per year. Conversely, the lack of effective treatment of bacterial diseases can be extremely costly. For example, nosocomial infections in North America cause over 20,000 direct and 80,000 indirect deaths, and their treatment costs more than $1.5 billion, whereas salpingitis due to the sexually transmitted pathogens *Neisseria gonorrhoea* and *Chlamydia trachomatis* has been estimated to be a $2 billion health care problem.

One example of a bacterium causing disease with substantial economic impact is *Pseudomonas aeruginosa*. It is responsible for approximately 11% of nosocomial infections in North America, and is a significant cause of fatal nosocomial pneumonias and terminal lung infections in patients with cystic fibrosis. Two CBDN researchers and a Canadian biotechnology company, Synthetic Peptides Incorporated (SPI), based in Edmonton, have developed a peptide that mimics the epithelial cell binding domain of the *P. aeruginosa* adhesin organelle, the pilus [1]. This peptide, when appropriately conjugated to a carrier, can be utilized as a vaccine which raises anti-adhesin antibodies that protect against infection. Through collaborations with other CBDN researchers in Calgary and Edmonton (Fig. 1), it has been found that this peptide may have value in raising anti-toxin antibodies in the one instance and anti-adhesin antibodies directed against major causative agents of gastrointestinal disease, as well as against certain fungi, in the other instance. Extensions of the original patent filing for the peptide vaccine have been sought for these new findings, and should enhance considerably the potential value of SPI's vaccine technology.

Different strategies based on adherence are being utilized by other CBDN researchers. For example, another Edmonton researcher, in collaboration with researchers at the Alberta Research Council, has developed a method for delivery of carbohydrate receptor mimics for bacterial toxins [2]. The carbohydrates, attached to a novel solid support

Table 1. Descriptions of project leaders' ongoing projects and their collaborations

Project leader	Location	Project description	Collaborators
Altman, E	N	Pseudomonas aeruginosa LPS structure/chemistry	G, N, V
Armstrong, GD	E	Novel anti-toxin therapy; receptor analogues	A, L, S[a]
Babiuk, LA[b]	S	Vaccines and adjuvants for food animals	E, S, N, V[a]
Beveridge, TJ	G	Bacterial ultrastructure; antibiotic action	C, E, G, L, V, B
Boissinot, M	L	Superoxide dismutases of bacteria	B, L, V
Bryan, LE	C	Antibiotic resistance in *H. pylori*; epidemiology	E, V
Chow, AW[b]	V	Toxic shock syndrome; superantigens	D, S, V
Clarke, AJ	G	Autolysins, β-lactamases and penicillin binding proteins	G, L, V[a]
Davies, J	V	Antibiotic resistance genes	B, G, V
Dillon, JR	D	Pathogenesis of anaerobic bacteria	E, L, V
Finlay, BB	V	[1]Invasion of host cells by intracellular bacteria [2]Live vaccine technologies	E, V
Hancock, REW	V	Compounds that overcome bacterial permeability barriers	B, C, E, G, N[a]
Hodges, RS[c]	E	Peptide conjugation technology; immunogenicity	C, E, S[a]
Irvin, RI	E	Cell binding domains of adhesins	E, V[a]
Iwama, GK	V	Stress, heat shock proteins and disease susceptibility in fish	B[a]
Kay, WW[b]	B	[1]Live attenuated vaccines for fish [2]Attenuated vaccine for *S. enteritidis*	G, V[a]
Lam, JS	G	Molecular studies on *P. aeruginosa* LPS	G, N, V
Levesque, RC[b]	L	[1]Structure: activity relationships amongst β-lactamases [2]*P. aeruginosa* genome sequencing project	C, E, V[a]
Lo, RYC	G	Molecular approaches to fish and cattle vaccines	B, G, N[a]
McBride, BC	V	Pathogenic factors of oral microbes	G, V
Mutharia, L	G	LPS immunochemistry of fish pathogens	B, G[a]
Nano, FE	B	*Chlamydia* LPS biosynthesis	G, S, V
Paranchych, W[b]	E	*P. aeruginosa* pilus vaccine	C, E, S, V[a]
Peppler, MS	E	*B. parapertussis* vaccine	E, D, N[a]
Perry, MB[b]	N	Klebsiella LPS chemistry	G
Potter, AA	S	Vaccines for food animals	E, N, S, V[a]
Schryvers, AB	C	Transferrin binding proteins; animal vaccines	G[a]
Sokol, PA[b]	C	*Y. enterocolitica* iron utilization	C, E, S, V
Speert, DP	V	Macrophage: bacterial interactions	B, G, V
Szalay, AA	E	Plant bacterial interactions; antibiotic action	B, E, V[a]
Taylor, DE	E	*Campylobacter* sp.; diagnostics; antibiotics	C, G[a]
Towers, N	V	Antibiotics from B.C. Native Indian medicinal plants	V
Trust, TJ	B	[1]S-layers of *Aeromonas* [2]*H. pylori* surface antigens	B, E, V[a]
Whitfield, C[b]	G	Molecular studies on bacterial polysaccharides	G, N
Woods, DE	C	*P. pseudomallei* vaccine	C, E[a]

[a] = Industry involved
[b] = Centre coordinator
[c] = Industry member
A = Alberta Research Council
B = Victoria, B.C.
C = Calgary
D = Laboratory Centre for Disease Control
E = Edmonton
G = Guelph
L = Laval
N = National Research Council
S = Saskatoon
V = Vancouver

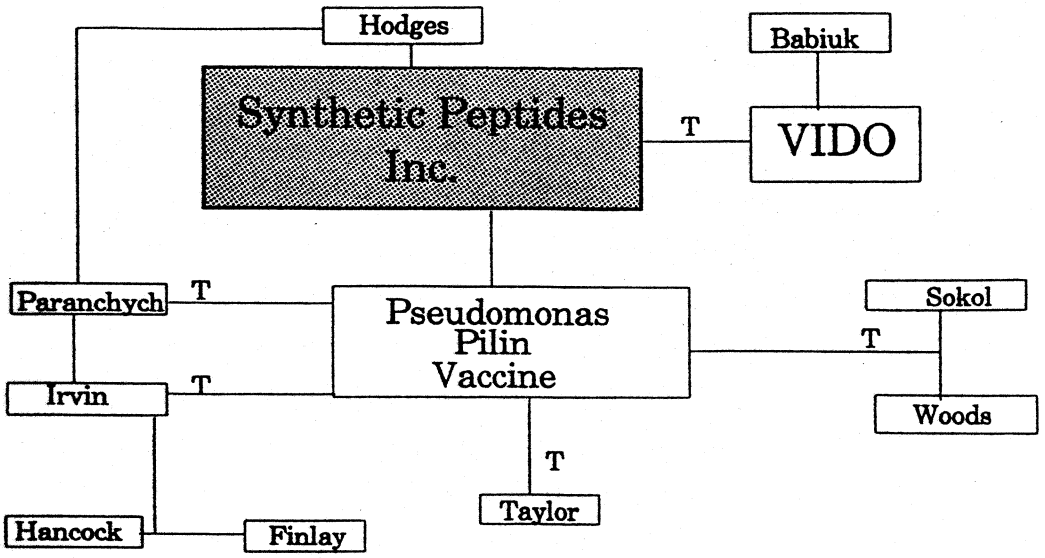

FIG. 1. An example of a series of Networking interactions in CBDN

(Synsorb) which comprises the porous shell-like structure of a diatom, are capable of binding the toxins, thus preventing the binding of toxins to their carbohydrate receptors and the resultant toxin-mediated disease symptoms. Synsorb technology is now being rapidly developed for use against verotoxic *E. coli* which cause hemolytic uraemic syndrome in children (as discovered by Canadian researchers A. Kharmali and collaborators). A phase two trial is being designed to start in 1993.

Two CBDN researchers in Vancouver, in collaboration with other Network researchers, are pursuing a generic strategy for development of diagnostics and vaccines, using a technique called epitope insertion. The basic technology, which has been filed for patent protection, involves mutagenizing the gene for *Pseudomonas aeruginosa* major outer membrane protein Opr F, by inserting 12 nucleotide stretches randomly throughout the gene. Successful insertions were identified as those capable of producing an intact Opr F protein, which could be exported to the outer membrane, expressed on the surface of *E. coli* and which remained reactive with a panel of Opr F-specific monoclonal antibodies. Such insertion mutants demonstrated 11 different sites capable of accepting an extra 4 amino acids, each marked by a unique restriction site in the 12 extra nucleotides of the mutated gene. An epitope, PNANPNA, from the malarial parasite major circumsporozoite protein, was cloned as a synthetic oligonucleotide into 9 of the sites and found using specific monoclonal antibodies to be expressed on the surface of *E. coli* in seven instances. It was found that between 18 and 69 extra amino acids could be accommodated at these sites. In addition, larger numbers of amino acids could be exported as fusion proteins, providing the amino terminal 153 amino acids of Opr F were present in the fusion protein. Permutations of this technology are now being developed to express random epitope libraries on the surface of *E. coli* (i.e. libraries of clones expressing every possible sequence of amino acids in a peptide inserted into Opr F), for use in identification of peptide mimics of epitopes useful in vaccines and diagnostics.

CBDN has also amassed considerable expertise in antibiotics research. CBDN researchers have demonstrated that, in the action of β-lactam antibi-

otics against Gram-negative bacteria, only 4 factors need to be considered [3]. These are the rate of permeation (P) across the outer membrane, the affinity (Km) of β-lactamase for the β-lactam, the stability (V max) of the complex of β-lactamase and the β-lactam, and the affinity (Si) of the target penicillin-binding proteins for the β-lactam. Indeed, it has been demonstrated that there is a precise mathematical relationship between these 4 factors and the MIC for the β-lactam. Two important resistance mechanisms are predicted by this work and indeed have been shown to be clinically important, namely β-lactamase overproduction and reduced outer membrane permeability. CBDN researchers are involved in devising methods for overcoming both resistance mechanisms. In the case of β-lactamases, CBDN researchers in Laval, Guelph, and Vancouver are using a combination of enzymology, site-directed mutagenesis, novel HPLC-based assays, and computerized molecular modelling techniques to study the determinants of interaction of β-lactamases with β-lactams and β-lactamase inhibitors. For outer membrane permeability, researchers in Vancouver have applied for a patent on a recombinant DNA technology for producing large amounts of peptide "permeabilizers", compounds which break down the outer membrane permeability barrier of Gram-negative bacteria.

Achievements to date

As discussed above, CBDN is only 28 months old. However, it has already made substantial progress towards its goals, progress which was assisted by a comprehensive review of its research program in January 1992. CBDN's primary aims include facilitating interactions with industry and working towards maximizing the dissemination of its research results to industry. Over time, it is believed that this will increase industry involvement in CBDN and dependence on government support in particular will decrease. The first concrete results of this approach are now evident. Seven research agreements have been finalized between pharmaceutical companies and CBDN. These should realize a projected income of approximately $1.2 million over the next 3 years. Not included in this estimate are those corporations who are contributing company resources which are estimated at just over $2 million in the same 3-year period. In addition, advanced negotiations are ongoing with 3 companies regarding major research collaborations funded by industry. One of the more important factors in generating interest from industry has been our ability to internally recognize and protect intellectual property. Indeed, more than 40 intellectual property disclosures to patent offices have been made to date and 25 have been or are in the process of being filed for patents. Identification and protection of ideas with commercial relevance prior to publication is facilitated by an active Working Group on Intellectual Property. This group's role is to sensitize the membership to issues of intellectual property, to review CBDN manuscripts for potential intellectual property value, and to review whether or not a specific discovery is of interest to a current or potential industry partner. The members all have experience interacting with industry and have signed separate confidentiality agreements.

As mentioned previously, CBDN has enjoyed considerable success to date; in fact CBDN researchers have over 150 manuscripts published or in press, and many more are in preparation. The Network is starting to receive international recognition. Currently CBDN is discussing collaborations with research groups in Hungary, Holland, Italy, and Australia. The next two annual CBDN meetings will be run in conjunction with a 1993 International Symposium on *Pseudomonas* and the 1994 Canadian Society of Microbiologists Annual Meeting. In addition, the management of CBDN is receiving many requests for information from overseas groups. Four vaccines developed in whole or in part through CBDN are being developed for commercialization. These include Synsorb and the *Pseudomonas* peptide vaccine described above, as well as vaccines for important fish and cattle diseases.

For CBDN to endure, it must continue to achieve two key points: strong operating cohesion and industry recognition worldwide. By doing this, CBDN will not only meet the Federal Government's objectives for the NCEs, but will surpass original expectations. As a rather unique organization in the business of science, CBDN is putting fundamental science to work.

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