microvascular endothelial cells serve as the major cell type providing the barrier and the non-endothelial cells, such as the perivascular glial cells, promote the formation of the barrier. Primary brain microvesicular endothelial cells (BMEC) co-cultured with astrocytes is the most common approach. Most researchers use bovine [3] or porcine [4] BMEC co-cultured with neonatal forebrain rat astrocytes or a glioma cell line [5].

An acceptable BBB model should have the following characteristics:

- tight cell–cell junctions to reproduce the tight cell barrier *in vivo*;
- active efflux transporters, especially Pgp, to model efflux of substrate molecules;
- active uptake transporters, such as glucose and amino acid transporters, to enable modeling of transporter-mediated drug uptake;
- expression of receptors as observed in BMEC *in vivo* to enable targeting the receptors to enhance drug permeability;
- expression of drug metabolizing enzymes (e.g. γ-glutamyl transpeptidase [6]) to model xenobiotic metabolism;
- similarity in responsiveness to permeation modulators as observed *in vivo* to enable investigation of disease-related or drug treatment-related changes in BBB functions.

Differences among species represent an aspect of the BBB that is often ignored, but one that I believe is important to the usefulness of an *in vitro* BBB model. There are many publications comparing *in vivo* BBB properties in one species (e.g. rat) with an *in vitro* model using cells from a different species (e.g. bovine or porcine), and then drawing conclusions on the utility of the *in vitro* system to predict BBB drug permeability for yet a third species (e.g. man) Although differences among species in drug metabolizing enzymes (e.g. P450 isoforms) have been well studied, the differences in BBB functions are yet to be clearly defined. It is not prudent to assume that such differences are not present or important, especially as there is evidence that illustrates differences among species in Pgp functions [7-9].

Development of a reproducible and practical BBB model that can accurately predict BBB permeability of drugs in humans is a research area that will enhance the efficiency of drug development. Careful definition of the BBB physiology and biochemistry, paying particular attention to crucial differences among species, will guide the development of the most appropriate experimental models.

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The development of antimicrobials and vaccines against bacterial bioterrorism agents – where are we?

Over the next five years, the US National Institutes of Health (NIH) plan to spend hundreds of millions of dollars to discover and develop or improve treatment and prevention modalities for agents of bioterrorism. The NIH has recently funded eight centers designated as 'Research Centers of Excellence.' These centers have been awarded $350 000 000 to develop new antibiotics and vaccines to protect the US population from agents of bioterrorism, as well as novel means to detect them [1]. Several other programs are currently under consideration and/or development at the NIH to further expand our scientific armamentarium against these agents. Similar funds are being spent to upgrade the sadly neglected public health infrastructure. The salient question is: will this strategy prove successful in protecting the US from future bioterrorism attacks?

In a recent edition of *Drug Discovery Today*, Greenfield and Bronze reviewed many of the key issues surrounding the prevention and treatment of three bacterial agents, *Bacillus anthracis*, *Yersinia pestis* and *Francisella tularensis*, as well as the toxins produced by *Clostridium botulinum*, which are believed to pose the greatest threat as bacterial bioterrorism agents [2]. The research needs for each one of these agents is clearly reviewed and should be an important part of the research agenda of these NIH research centers.

There is a need for clearer understanding of the efficacy of antimicrobial agents against *B. anthracis, Y. pestis* and *F. tularensis*. The strategy of using prophylactic antimicrobials for combating a bioterrorism attack with one of these three agents is well

described in the medical literature [3]. Unfortunately, naturally occurring and engineered antimicrobial resistance is a potential threat [2,4]. Therefore, our research agenda must include better means for detecting drug resistance in bioterrorism agents and improved animal models to assess efficacy of a wide array of currently available antimicrobial agents. We must also use our understanding of the molecular pathogenesis of these three organisms to identify potential targets that can be used in the development of novel antimicrobial agents. One of the challenges facing the development of these novel agents is the paucity of human disease caused by these organisms. This means human clinical trials will be limited primarily to safety, with understanding of efficacy being derived from animal models. With a limited understanding of the efficacy of these novel agents and concerns about their safety, how likely is it that these novel antimicrobials will be used as a primary line of defense against the targeted bioterrorism agents?

Vaccines are an attractive strategy to prevent infections in large populations. This approach tends to be much less expensive than antimicrobial prophylaxis and has proven time and again to be effective. The only human disease of note to be eliminated, smallpox, was accomplished with this strategy. Other viral diseases such as polio, measles, rubella and mumps have been eradicated from many regions of the world because of effective vaccines. A major challenge facing those attempting to make vaccines against these four bioterrorism agents is the difficulty of making an effective bacterial vaccine. There are currently four bacterial vaccines that offer excellent protection and a low rate of side effects: the conjugate vaccines for *Haemophilus influenzae* type b and *Streptococcus pneumoniae*, and the toxoid vaccines for *Corynebacterium diptheriae* and *Clostridium tetani.* However, attempts to develop vaccines against other bacteria have proven much less successful. An excellent example of the problems encountered in trying to produce a safe and efficacious vaccine against a bacterium whose pathogenesis is well understood and which possesses a virulence factor that can induce protective immunity is *Vibrio cholerae.* Several candidate vaccines have been developed over the past 25 years using state-of-the-art molecular genetic approaches [5]. None has yet approached the levels of safety and efficacy seen with the four previously described bacterial vaccines. Another problem is vaccine acceptance in at-risk populations. Although it would seem intuitive that individuals threatened with an infectious agent with a high mortality would readily accept vaccination against that agent, experience with influenza vaccine in the US would suggest otherwise. At best no more than two thirds of adults >65 years of age are vaccinated annually against influenza in the US despite the fact that this is one of the leading causes of preventable deaths in this age group [6]. It is unlikely that there would be a high level of acceptance of vaccines against any bioterrorism agent in the absence of an actual disease outbreak. As a result, a strategy needs to be developed to quickly vaccinate the population if one of these bioterrorism agents is released for which there is a vaccine. The need for multiple vaccine doses will further complicate this effort and should be considered during the development of the bioterrorism agent vaccines.

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