BIOLOGY OF CORONAVIRUSES 1980

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INTRODUCTION

It is well established that the large group of coronavirus induce, in a variety of hosts, a spectrum of acute diseases. As summarized in Table 1, it is shown that both murine and feline coronaviruses induce infection in several different organs of the host, when for example these agents lead in man, cattle, dogs or rats only to single disease manifestation. Current interest in the biology of coronaviruses centers around the mechanism of the pathogenesis as well as virus-host interactions which lead to subacute or chronic diseases.

CORONAVIRUS PERSISTENT INFECTION IN TISSUE CULTURE

This meeting provided an opportunity to review the characteristics of several different systems in which it has been established that persistent coronavirus infections can be induced in tissue cultures of one sort or another. Some of the main characteristics are set out in Table 2. It is not certain whether the systems described are really homogeneous and comparable. In other words, even when cloned cells and cloned viruses are used it is possible that there is more than one interaction that can take place and lead to the interaction for which we select, namely that the cells survive and divide and virus is shed. It is clear for instance that there may be only 1-20 % cells producing

Table 1. Spectrum of Diseases Caused by Different Coronavirus Groups

Virus	: MHV*	RCV	TBDV	TGEV	HEV	NCDCV	IBV	CCV	FCV	HCV	HECV
Host	: mouse	rat	turkey	pig	pig	bovine	chicken	dog	cat	man	man
TYPE OF DISEASE											
encephalitis	+				+				+		
enteritis	+		+	+	+	+		+	+		+
hepatitis	+								+		
adenitis	+										
nephritis	+			+			+		+		
pancreatitis	+										
peritonitis	+								+		
respiratory infections	+	+	+	+	+		+		+	+	

* Abbreviations according to Tyrrell et al., Intervirology, 5, 76, 1975.

virus antigens as judged by immunofluorescence; in some instances the other cells which are resistant have been cloned and found to be uninfected and susceptible and the system represents the outcome of some sort of cell coronaviruses and may indeed shed virus either on further culture or by fusion with susceptible cells. It is therefore important that in future the cultures are analysed at the cellular level by obtaining clones and studying them individually.

The viruses recovered from persistently infected cells seem very variable. The common idea that such cultures select ts viruses was not supported, though some showed small plaques, and in some, virulence for the animal host was also reduced. Nevertheless in one case there was increased virulence for the original host cells. There seems to be a need to look in more detail at the growth of these cells and to examine at the molecular level how cloned cells achieve an equilibrium with the virus. This will allow further biological investigation, but it cannot substitute for a study of experimental diseases in animals.

CORONAVIRUS INFECTION in vivo

A number of different model diseases have been described and particular attention directed to the sub-acute or chronic conditions, the implication being that we understand fairly well how acute infections occur and are dealt with. I found these interesting but I was concerned that so often experiments were evaluated solely as to whether an animal lived or died. This is a clear cut "all or none" phenomenon and can be readily determined without using special equipment, but death has a very important qualitative aspect too. We should know how the animal died. Pathological examination showed there were at least three different sorts of subacute disease of mice and rats, the demyelinating syndrome, meningo-ependymitis and generalised vasculitis (Virelizier, this volume). It is good that workers are trying to provide defined and reproducible models in this field too, and the use of cloned virus and mutants and of inbred strains of animals is valuable.

I am however rather unhappy with the references to genetic factors as though these were a separate

Table 2. Persistent Infections by Coronaviruses in Tissue Cultures

ESTABLISHMENT	Investigators (see this volume)				
	Chaloner-Larsson Johnson-Lussenburg	Hirano et al.	Holmes Behnke	Sorensen et al.	Stohlman- Frelinger (personal communication)
virus	229E	JHM	A 59	JHM/MHV3	JHM
host cell	L132	DBT	17 Cl1	RN 2-2	N ₂ A
reproducibility	15 %	100 %	100 %	100 %	100 %
time of appearance (passage No.)	3	15	1	1	3
PROPERTIES OF PERSISTENT CELL LINES					
percentage of cells expressing viral antigen (FA)	N.D. *	10-15 %	10-20 %	1 %	20 %
presence of infectious virus	+	+	+	+	+
resistance to superinfection by corona virus	+	+	+	+	+
other virus	-	-	-	-	-
PROPERTIES OF THE VIRUS					
temp. sensitive	-	-	+	-	-
altered plaque morphology	-	+	+	-	-
alteration in virulence	increased	reduced	reduced	N.D.	N.D.

* N.D. not done

aspect of biology. Both the host and the parasite are totally the expression of genes and gene products, and what we are talking about is genetic factors which are identifiable or genetically controllable situations that can be exploited in one way or another. It was mentioned for instance (Klenk) that avirulent influenza viruses in the chick embryo infect the endoderm because the haemagglutinin is cleaved by a trypsin-like enzyme in these cells and as a result particles become infectious, whereas in mesoderm or ectoderm the haemagglutinin is not cleaved and the virus particle may be formed but does not become infectious. The pathogenetic virus on the other hand, is cleaved in all three types of cells.

This is the best example of how a minor difference in the structure of a virus polypeptide modifies its interaction with host enzymes and thus reveals genetic and structural facts which we should otherwise know nothing about. This is a particular example of a general principle referred to by Dr. Bang and enunciated by plant pathologists to the effect that one can only recognize and study changes in virulence if there is a susceptible host to study and that corresponding to the genes in the pathogen that determine its pathogenicity are corresponding genes in the host that determine its susceptibility and resistance.

A virus produces a disease by performing a long series of functions, including entry into the host, absorption, replication, cytopathogenicity etc. and it is important to remember this. I am against using loose phrases such as saying a virus is "virulent". It is better to try to describe what it does or does not do, e.g. initiate infection, multiply or kill cells. The presentation by Andries (this volume) was a fine example of how exactly the course of a disease may be mapped out, so that one can trace a complete series of links of a causal chain from the death from malnutrition, via the loss of gastric motility back to the neurotoxic effect of a virus and its original entry into the animal. Compared with this the course of events in many experimental diseases is not well worked out and it is therefore difficult, if not impossible, to postulate the likely key stages in the disease process.

INDICATION FOR FUTURE RESEARCH

It seems to me that one should consider using the philosophy of the "T club" who under the guidance of Hershey in the early days of the exploitation of the bacteriophages decided to concentrate work on phage T2. Thus they were able to concentrate a wide range of special skills and difficult investigations on a limited but still very complicated problem, and to make more progress, in the sense of answering important questions, than by doing as much work on a number of different and less well defined experimental systems. There are dangers of course - one may miss something by narrowing one's view - but I hope workers will consider it seriously. It is important to consider not only the immediate experiments, but also the possibilities for the future -for example whether inbred strains of animals are available with a good background of information on genetic characteristics, transplantation antigens and so on and a wide range of immunological reagents. The mouse is an obvious suggestion, but the rat might be satisfactory. It would also be desirable to mimic a significant human or veterinary disease as closely as possible.

The communications provided a number of warnings against being too easily persuaded by "neat" single experiments on isolated cells or with single so-called specific immunological reagents. Dr. Bang admitted that all the conclusions he drew from the early experiments on MHV in mouse peritoneal macrophages had to be changed in the light of further experiments. The results may only show "significant" results if the conditions such as the dose of virus or the type of culture medium are tightly controlled. In vitro results may nevertheless be significant, for example in showing differences in the susceptibility of hepatocytes to hepatotropic viruses. In the subacute and chronic conditions it is important to assess fully the functioning of the immune system and there are many gaps in our knowledge. Antibody is found in the demyelinated brain tissue, but against what antigen is it directed and is it pathogenic or merely an epiphenomenon? The general MHV vasculitis is apparently due to circulatory immune complexes, but what is the antigen, and what type of antibody do they contain?

In conclusion it seems to me that this is a particularly valuable meeting in which molecular biologists, virologists and experimental pathologists have sat down together to hear the latest results and to see things

from each other's point of view.

I think all involved are most grateful to Professor ter Meulen and his staff for arranging it and running it so well. There is no doubt that we have all benefited and that our future research will be improved by the new ideas we are taking away and the personal contacts we have made.