Volume 63 January 1970

Section of Clinical Immunology & Allergy

President
Professor Sir Michael Woodruff FRCS FRS

Meeting April 14 1969

Immunosuppressive Effects in Infection

Dr M H Salaman
(*Department of Pathology, Royal College of Surgeons of England, London*)

Immunodepression by Mammalian Viruses and Plasmodia

Depression of immune responses by viruses is receiving increasing though much belated attention. Early reports (von Pirquet 1908, Bloomfield & Mateer 1919) were forgotten and the subject neglected till 1960 when Old and his colleagues noticed that the hæmolysin response to sheep erythrocytes (SE) was depressed in mice infected with Friend's leukæmogenic virus (FV), and Gledhill (1961) found that FV and Moloney virus both enhanced the pathogenicity of mouse hepatitis virus. Since then reports of immunodepression by a wide variety of viruses, both oncogenic and non-oncogenic, have appeared.

Non-oncogenic Viruses
Reports on immunodepression by these viruses are summarized in Table 1.

Measles: In 1908 von Pirquet noticed that the tuberculin reaction in tuberculous children became negative during an attack of measles, and that afterwards tuberculosis often advanced rapidly. Later work has confirmed his observations on tuberculin sensitivity by more sensitive methods (Mellman & Wetton 1963, Brody & McAlister 1964, Starr & Berkovitch 1964), and it has been shown (Smithwick & Berkovitch 1966) that measles virus, added to lymphocytes of tuberculin-positive patients, prevents the mitogenic response to tuberculin PPD, but not the response to phytohæmagglutinin (PHA).

Rubella: In infants with congenital rubella there is no gross defect of antibody formation, but their lymphocytes do not respond to PHA (Alford 1965, Bellanti *et al.* 1965, Soothill *et al.* 1966). Normal lymphocytes treated with rubella virus *in vitro* lose their PHA-responsiveness. Polio, mumps and Newcastle disease viruses have the same effect (Montgomery *et al.* 1967, Olson *et al.* 1967, Dent *et al.* 1968). Recent work suggests some caution in accepting these results on PHA-sensitivity. The reaction is dependent on concen-

Table 1
Depression of immune responses by mammalian non-oncogenic viruses

	Antibody production		*Delayed hypersensitivity to tuberculin*	*Mitogenesis in lymphocytes*	
Viruses	*Primary*	*Secondary*		*Normal lymphocytes plus phytohæmagglutinin*	*Immune lymphocytes plus antigen*
Measles			+	−	+
Rubella	−			+	+
Influenza	?		+		
Mumps				+	
Newcastle disease virus				+	
Junin	+	+			
Lymphocytic choriomeningitis virus	+	+			
Murine cytomegalovirus	+				

+, depression. −, not affected. Blanks, not tested

tration of PHA, and the tests should be repeated using a range of dilutions of the mitogen (Simons & Fitzgerald 1968).

Influenza: The observation by Bloomfield & Mateer (1919) that the tuberculin reaction became negative during an attack of influenza has not been repeated, but recently it was shown that the number of hæmolytic cells in the spleens of mice injected with sheep erythrocytes (SE) is reduced if the cells are preincubated with influenza virus (Mazzur & Paucker 1967).

Junin virus, which produces lymphocytopenia and hæmorrhages in both man and guinea-pig, depresses primary and secondary hæmagglutinin production to human erythrocytes in guinea-pigs, and reduces plateau antibody titres (Parodi *et al.* 1967).

Lymphocytic choriomeningitis virus (LCM): Mims & Wainwright (1968) found that when LCM was injected into adult mice it temporarily depressed antibody production to SE and to human serum albumin, and decreased systemic anaphylaxis to ovalbumin. Newborn mice inoculated with LCM showed a depressed antibody response for a few weeks, which returned to normal when they were adult. However, congenital carriers of LCM had normal immune responses throughout life. In adults, ectromelia was more lethal following an injection of LCM, and delayed hypersensitivity to ectromelia was reduced. Ectromelia and cowpox were not themselves immunodepressive.

Murine cytomegalovirus (CMV): In mice a small intravenous dose of this DNA virus is followed by an almost symptomless generalized infection. Virus can be recovered from the spleen for about a week, but may persist in the salivary glands. Antibody production to SE, and to Newcastle disease virus (NDV), was depressed by previous injection of CMV. In the latter case NDV multiplied in the spleens of mice for about four days, which it does not normally do. Interferon production was reduced for about the same time as immune responses (Osborn & Medearis 1967, Osborn *et al.* 1968).

We see, then, that immunodepression is not confined to one class of mammalian viruses. Wherever we have adequate data, immune depression is found to be approximately coterminous with systemic viral infection and, in the case of CMV and perhaps in others, with depression of interferon production.

As Table 1 shows, only a fraction of non-oncogenic viruses have been tested for immunodepression. It will not be surprising if the property is much more widespread than it now appears to be.

Oncogenic Viruses

It has been postulated that a degree of inadequacy of the immune system is a necessary condition for neoplastic growth (e.g. Prehn 1963). Physical and chemical carcinogens are known to have a profound and sometimes long-lasting immunodepressive effect (Ball *et al.* 1966, Stjernswärd 1965). It is clearly important to know whether immunodepressive action is a necessary property of oncogens, physical, chemical or viral.

Work on immunodepression by viruses which produce leukæmia in rodents antedates most of the reports on non-oncogenic viruses. Table 2 shows some general properties of the murine leukæmogenic viruses. There are two main subdivisions: (1) The Friend-Rauscher group, primarily affecting cells of the erythroid series, rapidly acting and equally pathogenic in adult

Table 2
Murine leukæmogenic viruses

Viruses	*Distribution*	*Pathology of overt disease*	*Age-susceptibility*
Friend (both anæmia and polycythæmia inducing types)	Latent in some wild and many laboratory mice	Rapid development (2–5 weeks). Splenomegaly, proliferation of erythroblasts and precursors, with anæmia or polycythæmia. Occasionally late malignant lymphoma	Adults as susceptible as newborns
Rauscher			
UL (a Rauscher-type virus isolated from urethane-induced leukæmia)			
Rowson-Parr virus (minimally pathogenic: a variant of Friend?)		No gross pathology	
Gross	Latent or overt in a few laboratory strains	Slow development (2–8 months). Enlargement of thymus, lymph nodes and spleen. Proliferation of malignant lymphoblasts or myeloblasts	Newborns more susceptible than adults
Moloney			

Table 3
Depression of immune responses by murine leukæmogenic viruses

	Antibody production		*Delayed hypersensitivity*		
Viruses	*Primary*	*Secondary*	*Tuberculin*	*Others*	*Graft rejection*
Friend	+ (early▲)	+		+	
RPV●	+ (early)	+			
Rauscher	+ (early)	+			
UL■	+ (early)				
Moloney	+ (late x)				
Gross	+ (late)				+

●Rowson-Parr virus, minimally pathogenic, a contaminant of Friend virus preparations
■Rauscher-type virus isolated from urethane-induced leukæmia
▲From 1st day after injection
x From about 4th week after injection

and newborn mice. (2) The Gross-Moloney group, primarily affecting lymphoid cells, slowly acting and more pathogenic in newborn than in adult mice. Immunological studies on these viruses are summarized in Table 3. The many blank spaces here, as in Table 1, show the large gaps in our knowledge.

Only one mammalian virus which produces solid tumours, murine sarcoma virus (Harvey 1964), has been tested for immunodepression. Despite its close links with the leukæmogenic viruses it was found to be inactive by the tests used (Wedderburn 1969). There is no convincing evidence of depression of antibody production by polyoma, the mammary tumour virus, Shope fibroma or Shope papilloma viruses, though defects in cellular immunity have not been excluded. But this field has been barely scratched.

Immunodepression by the lymphomagenic viruses Gross Passage A and Moloney is easily missed because, like their pathogenic effects, it develops slowly. No immune defects appear for at least three weeks after infection, in fact, in our hands, not before enlargement of lymphoid organs, i.e. 2–6 months after infection, depending on age at inoculation. However, Metcalf & Moulds (1967) found that mice of the naturally high-leukæmic strain AK, which carries the Gross virus, show a depression, slight in the pre-leukæmic, more severe in the leukæmic period; and Gross virus injected into infant C_3H mice is found to depress antibody response and delay rejection of homografts, about two months later (Peterson *et al.* 1963, Dent *et al.* 1965).

Friend and Rauscher viruses have a much more rapid pathological effect and their immunodepressive action, detectable almost immediately after infection, has been more fully studied (Salaman & Wedderburn 1966, Odaka *et al.* 1966, Siegel & Morton 1966*a*, *b*, Ceglowski & Friedman 1967, Chan *et al.* 1968, Wedderburn & Salaman 1968, Gelzer & Dietrich 1968). It may be significant that this group is fully pathogenic in adults, while the lymphomagens, whose immunodepressive effect develops much more slowly, must be injected into newborn animals to produce their full pathogenic effects.

I shall take some experiments with Friend virus as the paradigm of these studies. Differences within the group are minor. In this work Dr Wedderburn and Dr Bendinelli collaborated with me, and in some experiments we had the help of Dr Asherson and Dr Bainbridge (Salaman & Wedderburn 1966, Wedderburn & Salaman 1968, Bendinelli 1968, Bendinelli & Asherson 1968, Bendinelli & Bainbridge 1968).

Fig 1 shows the peak (fourth day) numbers of hæmolytic plaque-forming spleen cells after an injection of SE, as measured by the Jerne test, plotted against the interval between injection

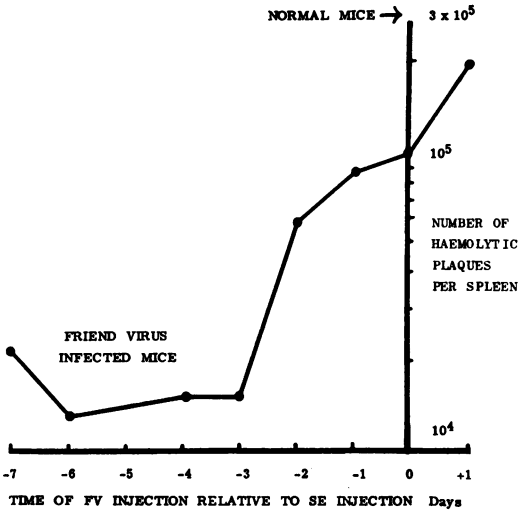

Fig 1 *Effect of Friend virus (FV) injected at various times on the number of hæmolytic plaque-forming cells (PFC) in the spleens of mice four days after the injection of sheep erythrocytes (SE)*

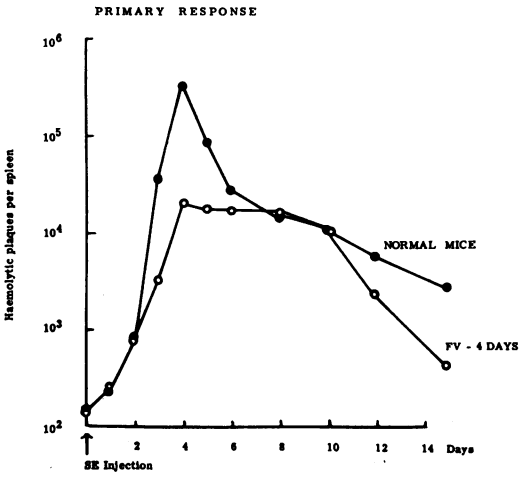

Fig 2 *Effect of FV injected four days before SE on the course of the primary PFC response.* ●——●, *normal mice.* ○——○, *FV-infected mice*

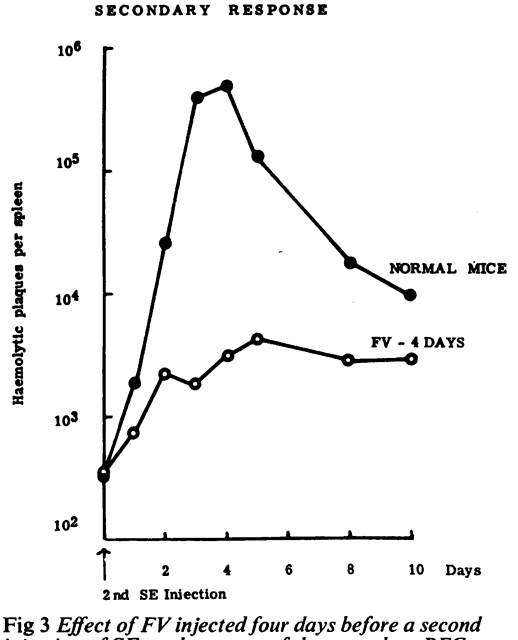

Fig 3 *Effect of FV injected four days before a second injection of SE on the course of the secondary PFC response.* ●——●, *normal mice.* ○——○, *FV-infected mice*

of FV and injection of the antigen. Slight depression is seen when virus and antigen are injected together, little or none when virus is injected after antigen, but profound depression when virus is injected three or more days before antigen. When virus precedes antigen by nine days or more, i.e. when gross splenomegaly has developed before antigen injection, there is a further depression of response.

When the number of hæmolysin-producing cells is followed daily after a primary injection of SE it is found that the difference between infected and normal mice is first seen after 48 hours (Fig 2). There appears to be an early stage of the primary response resistant to viral suppression. Injecting the virus ten days before antigen does not abolish this early resistance. In the secondary response there is no resistant phase: the response in infected mice is depressed from its inception (Fig 3). These figures record presumptive IgM antibody-producing cells. Presumptive IgG producers were also counted: they were even more severely suppressed than IgM in both primary and secondary responses, and in neither was there any early resistance.

Bendinelli & Asherson (1968) have studied delayed hypersensitivity. They found that when FV was injected before picryl chloride was applied to the abdominal skin, the reaction to a subsequent application to the ear was significantly reduced, and antibody to the picryl group was virtually abolished.

Bendinelli & Bainbridge (1968) studied the 'homing' of ^{51}Cr-labelled spleen cells from normal and from FV-infected mice when injected intravenously into normal or infected recipients. Fewer infected than normal spleen cells 'homed' to the spleen, but FV infection of the recipients made no difference.

Lately Dr Wedderburn and I have been interested in a virus which was found contaminating our stocks of FV (Rowson & Parr 1968). It produced a slight but persistent splenomegaly, and is strongly protective against subsequent infection by FV. There are no gross pathological changes, but the virus is recoverable from plasma and spleen for weeks or months. We have hitherto called this agent 'small spleen virus' (SSV), but this name is not satisfactory and will be superseded by 'Rowson-Parr virus' (RPV) (Rowson & Parr 1969). Dr Wedderburn has found that it has an immunodepressive effect against SE comparable, in the early stages of infection, with that of FV itself, and a lesser though detectable depression which persists for at least eight months. RPV has already proved a very useful tool in the study of resistance to other infections, and to the growth and metastasis of tumours.

Plasmodia

Last year Mr Denis Burkitt revived and developed the theory of Dalldorf *et al.* (1964) that the observed correlation between high incidence of his lymphoma and certain climatic conditions might be due to a predisposing role of chronic malarial infection (Burkitt 1968). It seemed possible to us that, if there were such a causal connexion, it might depend on an immunode-

pressive action of plasmodia. With the help of Professor P C Garnham and Professor L Bruce-Chwatt we examined this possibility, using the murine *Plasmodium berghei yoelii*, which produced a severe but self-limiting disease in Balb/c mice. With a suitable dose of this parasite the course of the overt disease is 14–17 days.

When SE were injected at the height of the disease, i.e. between the 9th and the 11th day after infection, there was a profound but short-lived depression in the number of hæmolytic spleen cells (Salaman *et al.* 1969).

We tried to find out whether this short immunodepression would affect the course of leukæmogenesis by viruses, and got an unexpected result. When the plasmodium was injected into apparently healthy mice carrying a virus of the Rauscher type by vertical transmission, the malarial disease was completely altered. Instead of recovering, as normal mice do, almost all the virus-carrying mice died in about three weeks, with extreme anæmia and 50–100% of their erythrocytes parasitized. The normal immune response to the plasmodium, which comes into effect about the 10th day, seemed to be absent. Thus there were virtually no survivors which could be observed for possible increased incidence of leukæmia later.

When RPV was injected into normal mice before, with or after the plasmodium the same thing happened: they nearly all died of fulminating malaria.

Evidently the short sharp immunodepression produced by the plasmodium itself does not prevent a subsequent immune reaction to the parasite, but a virus with a prolonged immunodepressive action converts this type of plasmodial infection from a self-limiting to a fatal disease.

The best animal model of the situation which Burkitt has suggested for man would be chronic plasmodial infection in a mouse carrying a leukæmogenic virus. We have failed to establish chronic infection with *Pl. berghei yoelii*. After recovery from a primary infection Balb/c mice appear solidly resistant to subsequent infection, and no further immunodepression occurs. However, Jerusalem (1968) has repeatedly infected Swiss mice with a plasmodium usually lethal in these mice, by cutting short the initial infection with a p-aminobenzoic acid-free diet. He found failure to reject foreign skin grafts at 11 days (personal communication), and after six months some evidence of increased incidence of malignant lymphoma.

Conclusion

The mechanism of immunodepressive action of viruses and of plasmodia is obscure. Antigenic competition is a distinct possibility, but the weight of evidence is against it. Although leukæmogenic viruses are in general strong stimulators of proliferation of certain cell types, they may possibly inhibit mitosis of antibody precursor cells. These and other possibilities will be examined in the future.

The implications of possible immunodepression by a wide variety of viruses and other infective agents are interesting and rather alarming. It may predispose to other infections, or increase their severity. It may also perhaps facilitate proliferation and metastasis of neoplastic cells.

REFERENCES

Alford C A (1965) *Amer. J. Dis. Child.* **110**, 455
Ball J K, Sinclair N R & McCarter J A (1966) *Science* **152**, 650
Bellanti J A, Artenstein M S, Olson L C, Buescher E L, Luhrs C E & Mitslead K L (1965) *Amer. J. Dis. Child.* **110**, 464
Bendinelli M (1968) *Immunology* **14**, 837
Bendinelli M & Asherson G L (1968) *A.R. Brit. Emp. Cancer Campgn* **45**, 137
Bendinelli M & Bainbridge D R (1968) *A.R. Brit. Emp. Cancer Campgn* **45**, 137
Bloomfield A & Mateer J (1919) *Amer. Rev. Tuberc.* **3**, 166
Brody J A & McAlister R (1964) *Amer. Rev. resp. Dis.* **90**, 607
Burkitt D P (1968) *J. nat. Cancer Inst.* **42**, 19
Ceglowski W & Friedman H (1967) *Proc. Soc. exp. Biol. (N.Y.)* **126**, 662
Chan G, Rancourt M W, Ceglowski W S & Friedman H (1968) *Science* **159**, 437
Dalldorf G, Linsell C A, Barhart F E & Martyn R (1964) *Perspect. Biol. Med.* **7**, 435
Dent P B, Olson G B, Good R A, Rawls W E, South M A & Melnick J L (1968) *Lancet* i, 291
Dent P B, Peterson R D A & Good R A (1965) *Proc. Soc. exp. Biol. (N.Y.)* **119**, 869
Gelzer J & Dietrich F M (1968) *Int. J. Cancer* **3**, 51
Gledhill A W (1961) *Brit. J. Cancer* **15**, 531
Harvey J J (1964) *Nature (Lond.)* **204**, 1104
Jerusalem C (1968) *Z. Tropenmed. Parasit.* **19**, 94
Mazzur S R & Paucker K (1967) *J. Immunol.* **98**, 689
Mellman W J & Wetton R (1963) *J. Lab. clin. Med.* **61**, 453
Metcalf D & Moulds R (1967) *Int. J. Cancer* **2**, 53
Mims C A & Wainwright S (1968) *J. Immunol.* **101**, 717
Montgomery J R, South M A, Rawls W E, Melnick J L, Olson G B, Dent P B & Good R A (1967) *Science* **157**, 1068
Odaka T, Ishii H, Yamaura K & Yamamoto T (1966) *Jap. J. exp. Med.* **36**, 277
Old L J, Clarke D A, Benacerraf B & Goldsmith M (1960) *Ann. N.Y. Acad. Sci.* **88**, 264
Olson G B, South M A & Good R A (1967) *Nature (Lond.)* **214**, 695
Osborn J E, Blazkovec A A & Walker D L (1968) *J. Immunol.* **100**, 835
Osborn J E & Medearis D N jr (1967) *Proc. Soc. exp. Biol. (N.Y.)* **124**, 347
Parodi A S, Nota N R, Guerrero L B de, Frigerio M J, Weissenbacher M & Rey E (1967) *Acta virol.* **11**, 120
Peterson R D A, Hendrickson R & Good R A (1963) *Proc. Soc. exp. Biol. (N.Y.)* **114**, 517
Pirquet C von (1908) *Dtsch. med. Wschr.* **34**, 129
Prehn R T (1963) *J. nat. Cancer Inst.* **31**, 791
Rowson K E K & Parr I B (1968) *Biochem. J.* **106**, 39P
(1969) *Int. J. Cancer* (in press)
Salaman M H & Wedderburn N (1966) *Immunology* **10**, 445
Salaman M H, Wedderburn N & Bruce-Chwatt L J (1969) *J. gen. Microbiol.* **58**, 666
Siegel B V & Morton J I (1966*a*) *Immunology* **10**, 559
(1966*b*) *Proc. Soc. exp. Biol. (N.Y.)* **123**, 467
Simons M J & Fitzgerald M G (1968) *Lancet* ii, 937
Smithwick F M & Berkovitch S (1966) *Proc. Soc. exp. Biol. (N.Y.)* **123**, 276
Soothill J F, Hayes K & Dudgeon J A (1966) *Lancet* i, 1385
Starr S & Berkovitch S (1964) *New Engl. J. Med.* **270**, 386
Stjernswärd J (1965) *J. nat. Cancer Inst.* **35**, 885
Wedderburn N (1969) PhD Thesis. London University
Wedderburn N & Salaman M H (1968) *Immunology* **15**, 439