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VACCINES USING INTERFERON AS AN ADJUVANT TO AN ANTIGEN

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(57) Claim

1. A vaccine comprising an antigen effective to promote specific immunity against an infectious organism or against a biologically active substance and as adjuvant an effective amount of interferon- γ .

22. A method for protecting hosts from infections by infectious organisms or for controlling the activity of a biologically active substance which comprises introducing into said host an effective amount of a vaccine as claimed in any one of claims 1 to 19.

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Complete Specification for the invention entitled:
"IMPROVEMENTS IN AND RELATING TO VACCINES"

The following statement is a full description of this invention,
including the best method of performing it known to us :-

The present invention relates to the production of novel vaccines and to the use of those vaccines. More specifically, this invention relates to vaccines, comprising at least two components, one of which
5 immunologically corresponds to a T cell and B cell determinant portion of an antigen and the other is an interferon which serves as an adjuvant.

Classically, a vaccine is manufactured by introducing a
10 killed or attenuated organism into the host to initiate the normal immune response to the organism while, desirably, avoiding the pathogenic effects of the organism in the host.

15 The infectious organism/antigen against which the vaccine according to the invention is effective may be selected from any class of infectious organism, for example bacteria such as E.coli, Corynebacterium diphtheriae, Salmonella typhi, Streptococcus
20 pneumoniae, Vibrio cholerae, Mycobacteriae, viruses such as hepatitis-, tetanus-, influenza-, polio-, papilloma-, foot and mouth-, rhino-, rubella-, measles-, mumps-, rabies-, varicella-, pertussis-, pox-, encephalomyocarditis- or retroviruses, protozoa
25 such as trypanosomes or plasmodia, helminths, fungi and other organisms parasitic in the host for whom the vaccine is intended.

Viral hepatitis continues to rank as one of the most
30 important unconquered diseases of mankind. The general term, viral hepatitis, refers principally to hepatitis B (serum hepatitis), although other known viruses and cytomegalovirus can cause hepatitis in man. Hepatitis is particularly known for its focal attack on the liver
35 but the disease also influences other organs.

In 1965, Blumber discovered an antigen circulating in the blood of certain human beings [J Am. Med. Assoc., 191, 541 (1965) and Ann. Int. Med., 66, 924 (1967)]. This substance was subsequently found by Prince to be
5 surface antigen of hepatitis B virus (HBsAg) that is produced in abundance by individuals who are chronically infected with the agent [Proc. Natl. Acad. Sci. (USA), 60, 814 (1968)].

- 10 There is an urgent need for a hepatitis B vaccine for groups which are at an increased risk of acquiring this infection. These groups include health care and laboratory personnel, and individuals requiring (1) maintenance hemodialysis; (2) repeated blood
15 transformations or the administration of blood products; (3) treatment with immunosuppressive or cytotoxic drugs and (4) treatment for malignant diseases and disorders associated with depression of the immune response. In addition, a vaccine is needed
20 for individuals living in certain tropical areas where hepatitis B infection is prevalent.

Current vaccines for HBV consist of subviral components of the virus surface coat (HBsAg) purified from the
25 plasma of chronically HBV-infected donors and inactivated [McAuliffe et al., Rev. Infect. Dis. 2, 470 (1980)]. Clinical trials have demonstrated the safety and efficacy of current HBsAg vaccines but such vaccines are limited in supply and are relatively
30 expensive, particularly for those countries with the highest incidence of HBV disease.

The invention concerns improvements in and relating to vaccines.

Malaria is a severe, widespread disease for which, despite years of extensive efforts, a vaccine has not been developed. See, for example, Science, Volume 226, page 679 (November 9, 1984). Experimentally, mammals, including man, have been protected against infection by the etiologic agent of malaria, Plasmodium, by vaccination with irradiated sporozoites. Clyde et al., Am. J. Trop. Med. Hyg., 24, 397 (1975) and Rieckman et al., Bull. WHO, 57 (Supp. 1), 261 (1979). Yoshida et al., Science, 207, 71 (1980) report that such protection is at least partially mediated by antibody directed against a protein on the surface of the sporozoite, the circumsporozoite (CS) protein; monoclonal antibodies raised against CS proteins neutralize infectivity in vitro and protect animals in vivo. The CS protein appears to be highly evolutionarily conserved within species, but is quite varied across species.

Various species of Plasmodium are known to infect man. These are P. falciparum, P. vivax, P. yoelii, P. ovale and P. malariae, the latter two occurring at much lower frequency. Other species of scientific interest are P. berghei and P. knowlesi, the host of these species being, respectively, rodents and monkeys.

Hopes are currently high that some form of human malaria vaccine will shortly be under test, but it will probably be a considerable time before a particular antigen, or even a single stage, emerges as the first choice (McGregor I. Parasitology Today; 1, 1-33 (1985)).

Rhinoviruses are RNA viruses and, according to the conventional classification of the viruses, they form a genus within the family of the Picorna viruses.

They are widespread, attack the upper respiratory tract in humans and cause acute infections which lead to colds, coughs, hoarseness etc. and are generally referred to as colds. Infections caused by rhinoviruses are among the most common sicknesses in man. Although the course of these diseases is generally harmless, colds do result in a temporary weakening of the organism. This may give rise to secondary infections caused by other viruses or bacteria which may, under certain circumstances, result in serious illness. In addition, the economic damage caused by rhinoviruses is considerable. It has been calculated that in the USA rhinovirus infections annually cause the loss of more than 200 million working days or school days. In addition, in recent years, there has been a considerable increase in rhinovirus infections in large conurbations. Whereas most other infectious diseases confer a permanent or longlasting immunity from the pathogen in question, infections caused by rhinoviruses may recur again and again. The reason for the absence of any lasting immunity is the large number of strains of rhinovirus. Hitherto, over 100 rhinovirus strains have been isolated, showing no or very few immunological cross-reactions with each other. After the infection has occurred, antibodies against the particular virus strain can be detected, but these confer no protection against other rhinovirus strains. Because of the large number of strains circulating in the population, repeated infections by rhinoviruses are possible.

One object of the EPA O 192 175 was therefore to prepare agents which give protection from infections caused by rhinoviruses.

These may then be used to stimulate an immune response directed against intact viruses. Studies of the use of oligopeptides for stimulating an immune response directed against polio viruses have been published

5 (Emini, E.A., Jameson, B.A. and Wimmer, E., Nature (London) 30, 699-703, (1983); similar investigations have also been carried out in the case of foot and mouth viruses (Bittle, J.L., Houghton, R.A., Alexander, H., Shinnick, T.M., Sutcliffe, J.G., Lerner, R.A.,

10 Rowlands, D.J. and Brown, F., Nature (London), 298, 30 - 33, (1982); Pfaff, E., Mussgay, M. Böhm, H.O., Schulz, G.E. and Schaller, H., EMBO J. 1, 869 - 874, (1982)).

15 Many gastro-enteric diseases in humans and animals, such as those caused by Escherichia coli and similar bacteria, are generally the result of toxin production which operates to disrupt the fluid balance in the

20 gastro-intestinal tract. The result is excessive production of fluids and electrolytes from the epithelial cells in the gastro-intestinal tract. (Moon, J.W., Adv. Vet. Sci. and Compt. Med., 18:179-211 (1974)). By way of example, certain strains of E.coli

25 cause cholera-like diseases in humans and young farm animals, which may result from the action of either of two toxins that have been isolated and identified as ST, which is heat stable, and LT, which is heat labile. (Kohler, E.M., Am. J. Vet. Res., 29:2263-2274, (1968):

30 Gyles, C.L. & Barnum, D.A., J. Inf. Dis. 120:419-426 (1968)).

Recent studies have shown that in order for pathogens to successfully colonize parts of the body, it is necessary for the pathogen to have the ability to adhere to the cell surfaces so that it can multiply.

5 After colonization, the pathogens produce agents, such as toxins, which cause the undesirable symptoms. (Science 209:1103-1106, Sept. 1980). The adhesins necessary for this adherence are typically structures called pili which are thread-like projections on
10 bacterial cell surfaces and are typically necessary if the pathogen is to cause disease.

15 It has been established that immunity to pathogens can be conferred by immunizing against the toxins which they produce; Dobrescu, L., and Huygelen, C., Zpl. Det. Med. B, 23:79-88 (1976), and by immunizing against the adhesin factor, Jones, G.W., and Rutter, J.M., Am. J. of Clinical Nutrition, 27:1441-1449, (Dec. 1974); Nagy, B., Infect. Immun., 27:21-24, (Jan. 1980). Vaccines
20 conferring such immunities have been prepared with killed pathogens which themselves contained genes for producing the offending substances. In addition, vaccines have been prepared using the pure adhesin
25 itself.

Many efforts are in progress to prepare vaccines for Acquired Immuno-deficiency Syndrome. Recently Capon et al. described molecularly cloned acquired
immunodeficiency syndrome polypeptides and a vaccine
30 against this syndrome which confers resistance to infection by acquired immunodeficiency syndrome associated retroviruses (EPA 01 87 041).

It is well known in the vaccine art that the administration of such antigens frequently results in relatively little immunity unless a further component, known as an adjuvant, is also present in the vaccine composition.

The vaccines with which the invention is concerned are primarily those which comprise an antigen effective to promote specific immunity against an infective organism, or against any biologically active substance whose activity is to be controlled (e.g. a hormone).

Many vaccines are complex and include not only the antigenic determinant of interest but many related and unrelated deleterious materials, any number of which may, in some or all individuals, induce an undesirable reaction in the host.

In the past, antigens have been obtained by several methods including derivation from natural materials, coupling of a hapten to a carrier and by recombinant DNA technology. Sela et al. [Proc. Nat. Acad. Sci. (USA), 68, 1450 (1971); Science, 166, 1365 (1969); and Adv. Immun., 5, 129 (1966)] have also described certain synthetic antigens.

Certain "synthetic" antigens have been prepared by coupling small molecules (for example, dinitrophenol) to carriers (such as bovine serum albumin), thus producing antigens which cause the production of antibody to the coupled small molecule. The carrier molecule is often necessary because the small molecule itself may not be "recognized" by the immune system of the animal into which it is injected. This technique

has also been employed in isolated instances to prepare antigens by coupling polypeptide fragments of known proteins to carriers, as described in the above-referenced Sela et al. articles.

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Chemically synthesized polypeptides offer considerable advantages in terms of cost and safety of vaccination programs.

- 10 It is known that antisera to synthetic polypeptides from the nucleotide sequence of various regions of the S gene of HBV react with native HBsAg by radioimmunoprecipitation [Lerner et al., Proc. Natl. Acad. Sci. (USA), 78, 3403 (1981)] and commercial
- 15 solid-phase radioimmunoassays for anti-HBsAg [Gerin et al., Viral Hepatitis, Szumess et al (eds.), 49-55 (1982)].

- 20 Before synthetic vaccines become an alternative to conventional ones ways must be found to increase the magnitude and duration of the immunity they confer. An advantage of attenuated viruses is that they keep proliferating and thus present ever more antigen to the immune system, thereby increasing the antibody level. A
- 25 synthetic peptide, like a killed virus, does not replicate, and so substances called adjuvants must be added to the vaccine to increase the level and duration of immunity.

- 30 Some highly effective adjuvants have been identified in animal tests, such as Freund's Complete Adjuvant, the dipeptide known as MDP, saponin, aluminium hydroxide, and Bordetella pertussis. However, such materials are not of proven efficacy in humans, for example Freund's Complete
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Adjuvant and saponin are liable to cause more or less severe ulceration at the site of injection, or other serious side effects, and are not acceptable for human pharmacology.

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One of the most powerful of these, Freund's adjuvant, is an emulsion of mineral oil and water mixed with killed bacteria (mycobacteria, a class that includes the agent of tuberculosis). Just how it works is not known.

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Powerful adjuvants, such as Freund's adjuvant, may not be administered to humans and instead, alum, or aluminium hydroxide, is commonly included with a killed-virus vaccine. The antigen is adsorbed onto the

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aluminium hydroxide particles and is released slowly after injection. In some cases, it appears alum will serve well enough with synthetic vaccines, but most such vaccines will require something better. Much research is currently aimed at the development of safe

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and effective adjuvants. Some hopeful results in this direction have been reported by Francois Audibert and Louis Chedid of the Pasteur Institute and Ruth Arnon and Michael Sela of the Weizmann Institute of Science, who recently elicited antibodies conferring passive immunity against the diphtheria toxin. They synthesized

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three peptides corresponding to regions of the toxin molecule. Before injection a synthetic peptide must be coupled to a carrier molecule. In some experiments the carrier was keyhole-limpet hemocyanin, a respiratory pigment of a mollusc which is often used for this purpose. The Pasteur-Weizmann group devised a

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carrier-adjuvant combination. They coupled their diphtheria toxin peptides to a carrier that had been linked to a simple derivative of the cell membrane derivative of mycobacteria cell membrane; the membrane derivative apparently serves as an adjuvant, significantly increasing the immunogenicity of the peptides.

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From the above it is clear that there is a need for a vaccine adjuvant capable of potentiating the immune response induced by an antigen without the untoward side-effects of many known adjuvants.

- 5 We have now found that interferon (IFN- γ) is an effective component which enhances immune response and thereby acts like an adjuvant. Thus one aspect of our invention provides IFN- γ for use as a vaccine adjuvant. Another aspect of our invention provides a vaccine comprising an
- 10 antigen effective to promote specific immunity against an infectious organism or against a biologically active substance and as adjuvant an effective amount of interferon- γ .

- A further aspect of the invention therefore provides a
- 15 vaccine against infection by an infectious organism, comprising an effective amount of an antigenic and immunogenic preparation of an infectious organism, for example bacteria, viruses, protozoa, helminths, fungi and other organisms parasitic in the host for whom the
- 20 vaccine is intended, an adjuvant interferon gamma, and a physiologically tolerable carrier and/or diluent if necessary, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said
- 25 antibodies immunoreacting with said infectious organisms and said vaccine protecting the host from infection.

- A still further aspect of the invention provides a vaccine against infection by viruses such as hepatitis-, tetanus-, influenza-, polio-, papilloma-, foot and
- 30 mouth-, rhino-, rubella-, measles-, mumps-, rabies-, varicella-, pertussis-, pox-, encephalomyocarditis- or retroviruses comprising an effective amount of a polypeptide that immunologically corresponds substantially to portions of the virus, as adjuvant



interferon gamma and a physiologically tolerable carrier and/or diluent if necessary, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said virus and said vaccine protecting the host from said viral infection.

A yet further aspect of the invention provides a vaccine against infection by protozoa such as trypanosomes or plasmodia comprising an effective amount of an antigenic and immunogenic preparation of the infectious organism, as adjuvant interferon gamma, and a physiologically tolerable carrier and/or diluent if necessary, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said protozoon and said vaccine protecting the host from said protozoa infection.

A still further aspect of the invention provides a vaccine against infection by bacteria such as E.coli, Corynebacterium diphtheriae, Salmonella typhi, Streptococcus pneumoniae, Vibrio cholerae or Mycobacteriae comprising an effective amount of an antigenic and immunogenic preparation of the infectious organism, as adjuvant interferon gamma and a physiologically tolerable carrier and/or diluent if necessary, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said



antibodies immunoreacting with said bacterium and said vaccine protecting the host from said bacterial infection. Preferred vaccines protect the host from bacterial infections causing gastro-enteric disease.

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The invention further comprises methods of preparing vaccines according to the invention and kits for said vaccines.

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A yet further feature of the present invention provides the use of interferon_γ for the preparation of vaccines for protecting hosts from infections by infectious organisms and a method for protecting hosts from infections by infectious organisms which comprises introducing into said host an effective amount of vaccine.

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For use in a vaccine intended for administration to humans, the interferon_γ is most preferably human interferon_γ produced by recombinant DNA techniques; such material is now commercially available; for example from Boehringer Ingelheim. For veterinary use, the interferon is preferably more or less closely related to the interferon found naturally in the species for whom the vaccine is intended, although again it will generally be of recombinant origin.

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The infectious organism or antigen can be further purified such as by addition of a selective precipitating agent, followed by a final chromatographic step such as ion exchange chromatography or reverse phase HPLC.

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In the vaccine of the invention, an aqueous solution of the infectious organism, preferably buffered at physiological pH, can be used directly. Alternatively, the polypeptide can be encapsulated within microparticles such as liposomes. It is also possible to use combinations of various infectious organisms in one vaccine.



The amount of antigen present in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific antigen is employed and whether or not the vaccine is further adjuvanted. Generally, it is expected that each dose will comprise 1 - 1000 µg of antigen, preferably 10 - 200 µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects.

~~The adjuvant interferon may e.g. be of the alpha, beta or gamma type.~~ We have noted a particularly strong adjuvant effect with gamma interferon. The amount of interferon required to produce the desired adjuvant effect should be found by experiment, but typically may lie in the range 100 - 50,000 units, e.g. 1,000 - 10,000 units per dose of vaccine. It is preferred to include the interferon_γ in the vaccine composition. Alternatively the interferon and antigen may be separately administered, preferably to the same site, in such manner that the desired adjuvant effect is obtained. For this purpose the antigen and adjuvant may be formulated in a two-part pack, e.g. in separate ampoules or the like. It is believed that the adjuvant effect of the interferon_γ is particularly valuable in the case of the first dose of vaccine to be administered.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A., 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877.



Vaccines according to the invention may be formulated in conventional manner, e.g. with sterile physiologically compatible carriers such as saline solution, excipients, other adjuvants, preservatives, stabilisers, and the like. Injectable preparations will normally be formulated to be substantially isotonic. Vaccines may be in liquid form, or may be in dry form for dissolution in a sterile carrier prior to use. Vaccines may be single-dose preparations, or may be in other forms such as multi-dose flasks for use in mass vaccination programmes. Preparations effective by subcutaneous administration will often be preferred on account of their ease of use, although other administration routes such as intravenous, intramuscular and intradermal are also contemplated.

~~When the adjuvant is alpha or beta interferon, oral vaccines may in some cases be effectively formulated, since these interferons are acid-stable.~~

If necessary, following an initial vaccination, subjects will preferably receive a boost after about 4 weeks, followed by repeated boosts every six months for as long as a risk of infection exists. The infectious organism against which the vaccine is effective may be selected from any class of infectious organism. An infectious organism of particular interest and importance is Plasmodium spp, e.g. the human malaria parasite P. falciparum and the lethal rodent parasite P. yoelii. In tests with vaccines for the latter organism in mice we have shown, as is demonstrated in the tests below, that IFN-gamma not only produces a powerful adjuvant effect, superior even to that given



by B. pertussis, but promotes a more rapid immunological reaction to infection such that an aparasitanaemic condition is achieved far more rapidly than by use of the known adjuvants.

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Since there is at present no convenient way to purify antigens by virtue of their T-cell stimulating properties, we have adopted the alternative strategy of first obtaining a highly protective soluble antigen preparation, and then testing the protective effect of individual components, without reference to their reactivity with antibodies. Once an antigen is isolated, its reactivity with both T and B cells can be evaluated and correlations with protectivity looked for.

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15 In the present invention we describe a simple and reliable soluble vaccine giving effective protection against the highly virulent YM strain P. yoelii. Given intraperitoneally with saponin it is somewhat less effective than a formalin-fixed parasite vaccine given intravenously with B. pertussis, which requires only one rather than two injections (Playfair et al., Immunology, 33, 507-515 (1977)). However the soluble vaccine is also quite effective when given

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25 subcutaneously or intramuscularly, which is of obvious relevance to human vaccination. Moreover, a comparison of the dose-response relationship given in Table 1 with that found for the 230,000 MW purified P. yoelii protein (Freeman & Holder, Clin. exp. Immunol., 54, 609-616 (1983)) both of which begin to lose full protectivity

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in the 1-2.5 µg range, suggests that the 230,000 MW protein is not the only protective antigen in our lysate.

An encouraging feature of these experiments has been the potency of recombinant IFN- γ as an adjuvant for subcutaneous use; this appears to be the first report of the ability of IFN γ to act as an adjuvant by enhancing priming. In this aspect IFN seems to stimulate immunological memory, i.e. using the conceptional framework of the clonal selection theory (Burnet, F.M., The Clonal Selection Theory of Acquired Immunity. Nashville Tn.; Vanderbilt University Press, 1959) this effect could be explained as if virgin T- and B-cells are stimulated by IFN to multiply and differentiate into memory cells. Therefore the term "adjuvant" according to the invention only stands for describing an effect which could be explained as if immunological memory is enhanced but neither this explanation nor the term adjuvant should in any way be used to limit the present invention.

For convenience in describing the degree of protection in 250 mice we have scored protection by vaccines as "early" or "late", since, in this experimental model, challenged mice either recover on days 8 - 10 (early) or between 15 - 20 (late). Table 1 shows all the protection data. Vaccinated mice given antigen (lysate) alone showed only occasional late protection, the subcutaneous route being somewhat better than intraperitoneal. As described earlier (Playfair & De Souza, Parasite Immunol., 8 409, (1986)) antigen i.p. plus saponin protected all the mice almost invariably by day 10.

The adjuvant effect of γ -IFN was only slightly inferior to that of saponin. With 5000 u, the dose used in most experiments, over 90% of the mice were protected including over 70% which recovered early: the intraperitoneal and subcutaneous routes were equally effective. Intradermal and intramuscular injection were also effective but somewhat less so. In a smaller series of experiments doses of IFN γ down to 200 units were also effective.



Table 1. Effect of adjuvants on protection

Vaccine	Adjuvant	Dose	Route	Recovered			Per cent protected
				Early	Late	Died	
—	—	—	—	0	0	32	0
<i>P. yoelii</i> lysate	—	25 µg	s.c.	0	4	28	12.5
	—	—	i.p.	0	0	19	0
	—	—	i.m.	0	2	12	14.3
	Saponin	25 µg	s.c.	7	2	2	82
			i.p.	36	2	0	100
	γ-IFN	5000 u	s.c.	25	6	3	91
			i.p.	14	2	2	89
		2000 u	s.c.	2	2	1	80
			s.c.	2	2	0	100
		1000 u	s.c.	5	4	3	75
			i.p.	2	3	0	100
		500 u	s.c.	3	1	1	80
			i.p.	4	0	1	80
		200 u	s.c.	2	1	2	60
			i.p.	1	3	1	80
		100 u	s.c.	1	0	4	20
			i.p.	3	0	2	60
	LPS	20 µg	s.c.	0	3	4	43
		2 µg	s.c.	0	3	3	50
		200 ng	s.c.	1	2	7	43
		20 ng	s.c.	1	1	4	33
		2 ng	s.c.	0	0	3	0
		200 pg	s.c.	0	0	2	0
—	Saponin	25 µg	i.p.	0	0	6	0
—	γ-IFN	5000 u	s.c.	0	0	6	0
—			i.p.	0	0	6	0

Mice were given two injections 2 weeks apart of vaccine and adjuvant by the route indicated. The results are the numbers of mice which cleared their parasitaemia early (< 10 days) or late (> 10 days) or died, following a challenge of 10⁴ parasitized red cells. The total per cent of mice protected is also shown.

An important consideration with all recombinant materials is whether the effects are due to contaminating endotoxin, particularly since lipopolysaccharide (LPS) is known to have adjuvant activity. We do not believe that the effects reported here are due to LPS since the endotoxin content (by Limulus assay) of the IFN was less than 0.25 EU/mg protein, which is equivalent to less than 0.1 pg per 5000 units of γ -IFN. As Table 1 shows, doses of LPS well above this range had only weak adjuvant activity in our model and never induced strong early protection. Saponin and γ -IFN alone were not protective, so it is unlikely that the γ -IFN is acting directly in the blood-stage parasites as has recently been shown for the liver stage (Ferreira et al., Science, 232, 881 (1986)).

While the above fully describes the invention and all preferred embodiments thereof, it is to be appreciated that the invention is not limited to the embodiments particularly described but rather includes all modifications thereof coming within the scope of the following claims.

Throughout this application, the terms "peptide" and "polypeptide" are used interchangeably. As used herein, the term "synthetic polypeptide" means a chemically synthesised polypeptide.

Throughout the application, the phrase "immunologically corresponds substantially to" is used herein in relation to the antigens to mean that the antigen induces production of antibodies that bind to the antigen and

(a) bind to the antigenic determinant of the native infectious organism or (b) induce T cell proliferation. Thus, the antigens of this invention function immunologically as do the corresponding infectious organisms whilst also being capable of inducing the production of antibodies to themselves.

The word "antigen" has been used historically to designate an entity that is bound by an antibody and to designate the entity that induces the production of the antibody. More current usage limits the meaning of antigen to that entity bound by an antibody, while the word "immunogen" is used for the entity that induces antibody production. In some instances, the antigen and immunogen are the same entity. Where an entity discussed herein is both immunogenic and antigenic, it will generally be termed an antigen or infectious organism.

The following examples are intended to illustrate the invention more fully without restricting it and should be read in conjunction with the accompanying Figures whose significance is explained in more detail hereinafter.

Legends to the Figures:

Figure 1: Effect of adjuvants on the priming of T-helper cells to P. yoelii antigens in vaccinated mice. Mice were bled 10 days after infection. Doses were: P. yoelii lysate 25ug, saponin 25ug, & -IFN 5000 units. Five to ten mice per group. Asterisks denote values significantly different (*P<0.02, **p<0.005) from the corresponding group without adjuvant.

Figure 2: Effect of adjuvants on the DTH (delayed type hypersensitivity) response to P. yoelii antigens in vaccinated mice. Five to ten mice per group. Details and significance value as Fig. 1.

Figure 3: Effects of adjuvants on the antibody response during a P. yoelii infection in vaccinated mice. Details and significance values as in Fig. 1.

Example 1

Mice

5 (C57 B1 x Balb/c) F1 mice of both sexes were used at 10-14 weeks of age.

Parasites

10 The virulent YM line of P.yoelii (A.A. Holder and R.R. Freeman, Nature 294, 361-364) was maintained by weekly blood passage. Mice were infected by i.v. injection of 10^4 parasitised red cells. Parasitaemias were counted on Giemsa-stained tail blood films.

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Preparation of vaccines

Donor mice infected i.v. with 10^7 parasitised red blood cells were bled 4 days later into heparinized phosphate buffered saline (PBS), when their parasitaemias were approaching 100%, 90% of the parasites being schizonts. After three washes in PBS the parasitised blood was lysed in 0.01% saponin at 37°C for 30 min and washed three times or until the supernatant was free of visible haemoglobin. The pellet was resuspended in extraction buffer (see below) at a ratio of 1 vol pellet to 5 vols buffer. Solubilization was carried out at 4°C for 3 hrs with vortexing at intervals. Cell debris and insoluble material were removed by microfuging for 10 mins. The supernatant was then dialysed overnight against PBS before being used to immunise mice as described below.

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Extraction buffer

Triton X-100 was used at 0.5%, made up in 50 mM
Tris-HCl at pH 8.0, with 5 mM EDTA, 20 mM

5 iodoacetamide, 5 mM PMSF, 1 mcg/ml pepstatin (all from
Sigma) and 5 mcl/ml Trasylol, as described by Deans et
al (Clin. Exp. Immunol. 49, 297-309 (1982)), with the
addition of 2mcg/ml of leupeptin (Calbiochem).

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Vaccination and adjuvant

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Mice were vaccinated with two intraperitoneal
injections of 25 mcg of lysate protein at 2-week
intervals, either with no adjuvant (control), or with
Bordetella pertussis (10^8 organisms) or murine

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IFN-gamma (batch 3209-14 and 3209-33, (specific activity
 1.5×10^7 U/mg, purity > 99%, endotoxin content <
0.25 EU/mg) supplied by Boehringer Ingelheim. We also
investigated other routes of injection, namely
subcutaneous, intramuscular and intradermal.

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The mice were challenged three weeks later with 10^4
parasites i.v. The results are shown in Table 2,

Table 2

Effect of route and adjuvant on protection by a
Triton X-100 P.yoelli lysate

5	Adjuvant	Route	Recovered		Died	% protected
			Early	Late		
10	IFN-gamma 5000 units	i.p.	4	2	0	90
		s.c.	15	4	0	
		i.m.	2	4	2	
		i.d.	2	3	2	
15	B.pertussis 10 ⁸ organisms	s.c.	4	4	2	75
		i.m.	4	3	1	
		i.d.	2	4	4	
20	nil	i.p.	0	2	2	17
		s.c.	0	3	17	
		i.m.	0	2	9	
		i.d.	0	0	6	
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Patterns of recovery in vaccinated mice

All unvaccinated mice were dead by day 9. Vaccinated mice fell into two clear groups: those which recovered by day 7-10 and those which recovered around day 17-20. The latter group always showed a fall in parasitaemia on day 6 but failed to maintain this. For the purposes of the present study, we have therefore classified protection by vaccines as 'early' if parasitaemia was permanently cleared on or before day 10, and 'late' if it occurred subsequently. In fact 'late' recovery in these experiments almost invariably occurred between days 17 and 25.

It will be seen that IFN-gamma as adjuvant gave excellent protection, especially by the desirable s.c. route. Moreover 15 out of 19 mice in this group recovered early, whereas in the corresponding test with B. pertussis adjuvant 4 mice recovered early, 4 recovered late and 2 died. Without adjuvant, 17 out of 20 mice died. Further trials (results not shown) indicated that the adjuvants alone gave no protection.

25 Example 2:

Assay for T help

Mice were vaccinated as described in Example 1, and 3 weeks after the last dose of vaccine they were injected intravenously with 10^5 red blood cells parasitized with P. yoelii, coated with the hapten trinitrophenol (TNP) by incubation with 2,4,6-trinitrobenzene sulphonic acid (Playfair, De Souza & Cottrell, Immunology, 32, 681 (1977)) (TNP-PY), or with TNP-coated normal mouse red blood cells (TNP-MRBC). Four days later their spleens were assayed for direct

anti-TNP plaque-forming cells (PFC) in Cunningham chambers (Playfair et al., Immunology 32, 681 (1977)). Because we had previously shown that T helper cell priming is maximal with small doses of vaccine, we routinely used a single injection of 1 µg of antigen. Results are shown in Figure 1 which indicates that both saponin and γ-IFN induced excellent T helper priming. In this case γ-IFN was equally effective by the subcutaneous and intraperitoneal routes.

Example 3

Assay for delayed hypersensitivity

Mice were vaccinated as described in example 1, and 3 weeks after the last dose of vaccine they were injected in the pinna of the right ear with 3×10^6 red blood cells parasitized with P.yoelii in 10 µl of phosphate buffered saline (PBS) and in the left (control) ear with PBS only. One day later they were injected intravenously with 10^7 ^{51}Cr -labelled normal syngeneic bone marrow cells and after another 24 h they were killed, their ears were cut off and their radioactivity was counted on an LKB Gamma counter (Cottrell, Playfair & De Souza, Clin. exp. Immunol., 34, 147 (1978)). The results shown in Fig. 2 are the percentage of the total injected bone marrow cells homing specifically to the antigen-challenged ear, calculated as:

$$(\text{ct/min in right ear}) - (\text{ct/min in left ear})$$

x 100

$$\frac{\text{Total ct/min in bone marrow injection}}{\text{Total ct/min in bone marrow injection}}$$

Mice vaccinated with antigen alone gave DTH responses no stronger than unvaccinated mice, but both saponin and γ -IFN, given subcutaneously, induced strong priming for DTH. Saponin i.p. (the most protective combination) induced the strongest DTH but, surprisingly, γ -IFN by this route did not induce significant DTH.

Example 4

Antibody response

Antibody responses following challenge were measured by indirect fluorescence (IFA) using the slide method of Voller & O'Neill Bulletin of the World Health Organization, 45, 524 (1971). In vaccinated mice, this assay detects predominantly IgG antibody (Playfair & De Souza, Parasite Immunol. 1 197 (1979)).

Figure 3 shows the total IFA titres 10 days after challenge. Mice vaccinated with antigen alone had titres of 1/256-1/1000 whilst both saponin and γ -IFN boosted these 8-16 fold. Intraperitoneal injections appeared marginally better.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A vaccine comprising an antigen effective to promote specific immunity against an infectious organism or against a biologically active substance and as adjuvant an effective amount of interferon- γ .
2. A vaccine as claimed in claim 1 against infection by an infectious organism parasitic in the host, comprising an effective amount of an antigenic and immunogenic preparation of the infectious organism, interferon γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into the host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said infectious organism and said vaccine protecting the host from infection.
3. A vaccine as claimed in claim 2 wherein the infectious organism is a virus.
4. A vaccine as claimed in claim 2 wherein the infectious organism is a protozoon.
5. A vaccine as claimed in claim 2 wherein the infectious organism is a bacterium.
6. A vaccine as claimed in claim 3 against infection by hepatitis B virus comprising an effective amount of a polypeptide that immunologically corresponds substantially to portions of the hepatitis B virus surface antigen, interferon γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies



and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said hepatitis B virus and said vaccine protecting the host from hepatitis B viral infection.

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7. A vaccine as claimed in claim 3 against infection by tetanus-virus comprising an effective amount of an antigenic and immunogenic preparation of the tetanus-virus, interferon_γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said tetanus-virus and said vaccine protecting the host from tetanus-viral infection.

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8. A vaccine as claimed in claim 3 against infection by influenza virus comprising an effective amount of a polypeptide that immunologically corresponds substantially to portions of the influenza virus, interferon_γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said influenza-virus and said vaccine protecting the host from influenza viral infection.

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9. A vaccine as claimed in claim 3 against infection by rhinovirus comprising an effective amount of a polypeptide that immunologically corresponds substantially to portions of the rhinovirus, interferon_γ as an adjuvant and a physiologically

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tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said rhinovirus and said vaccine protecting the host from rhino-viral infection.

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10. A vaccine as claimed in claim 3 against infection by polio virus comprising an effective amount of a polypeptide that immunologically corresponds substantially to portions of the polio virus, interferon_γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said polio virus and said vaccine protecting the host from polio viral infection.

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11. A vaccine as claimed in claim 3 against infection by papilloma virus comprising an effective amount of a polypeptide that immunologically corresponds substantially to portions of the papilloma virus, interferon_γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said papilloma virus and said vaccine protecting the host from papilloma viral infection.

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12. A vaccine as claimed in claim 3 against infection
by retro viruses comprising an effective amount of
a polypeptide that immunologically corresponds
substantially to portions of the retro virus,
interferon^{-γ} as an adjuvant and a physiologically
tolerable carrier and/or diluent, said vaccine when
introduced into a host, being capable of inducing
the production of antibodies and stimulating B-
and/or T-cells in the host, said antibodies
immunoreacting with said retro viruses and said
vaccine protecting the host from retro-viral
infection.

13. A vaccine as claimed in claim 4 against infection
by Plasmodium sporozoites comprising an effective
amount of an antigenic and immunogenic preparation
of the Plasmodium organism, interferon^{-γ} as an
adjuvant and a physiologically tolerable carrier
and/or diluent, said vaccine when introduced into a
host, being capable of inducing the production of
antibodies and stimulating B- and/or T-cells in the
host, said antibodies immunoreacting with said
Plasmodium sporozoites and said vaccine protecting
the host from Plasmodium infection.

14. A vaccine as claimed in claim 4 against infection
by trypanosomes comprising an effective amount of
an antigenic and immunogenic preparation of the
trypanosome organism, interferon^{-γ} as an adjuvant and
a physiologically tolerable carrier and/or diluent,
said vaccine when introduced into a host, being
capable of inducing the production of antibodies



and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said trypanosomes and said vaccine protecting the host from trypanosome infection.

15. A vaccine as claimed in claim 5 against bacterial infection comprising an effective amount of an antigenic or immunogenic preparation of the pathogenic bacterium, interferon- γ as an adjuvant and a physiologically tolerable carrier and/or diluent, said vaccine when introduced into a host, being capable of inducing the production of antibodies and stimulating B- and/or T-cells in the host, said antibodies immunoreacting with said pathogenic bacterium and said vaccine protecting the host from bacterial infection.
16. A vaccine as claimed in claim 15 against gastroenteric disease.
17. A vaccine as claimed in one of the preceding claims wherein the interferon is human interferon- γ .
18. A vaccine as claimed in one of the preceding claims wherein the interferon- γ is produced by recombinant DNA techniques.
19. A vaccine according to any one of claims 1 to 18 substantially as described herein with reference to any of Examples 1 to 4 and Figures 1 to 3.
20. A method of preparing a vaccine as claimed in any one of the preceding claims comprising providing an effective amount of an antigenic and immunogenic preparation of the infectious organism and interferon- γ as an adjuvant and dissolving or dispersing an effective amount of said components in a physiologically tolerable carrier and/or diluent.



21. A method of preparing a vaccine as claimed in claim 20 wherein the effective amount of the antigenic and immunogenic preparation of the infectious organism and the effective amount of interferon- γ as an adjuvant are separately dissolved or dispersed in an effective amount of a physiologically tolerable carrier and/or diluent and both components are mixed just before injection or are separately administered.
22. A method for protecting hosts from infections by infectious organisms or for controlling the activity of a biologically active substance which comprises introducing into said host an effective amount of a vaccine as claimed in any one of claims 1 to 19.

Dated this 26th day of November, 1990,

BOEHRINGER INGELHEIM INTERNATIONAL GmbH,
By its Patent Attorneys,
DAVIES & COLLISON

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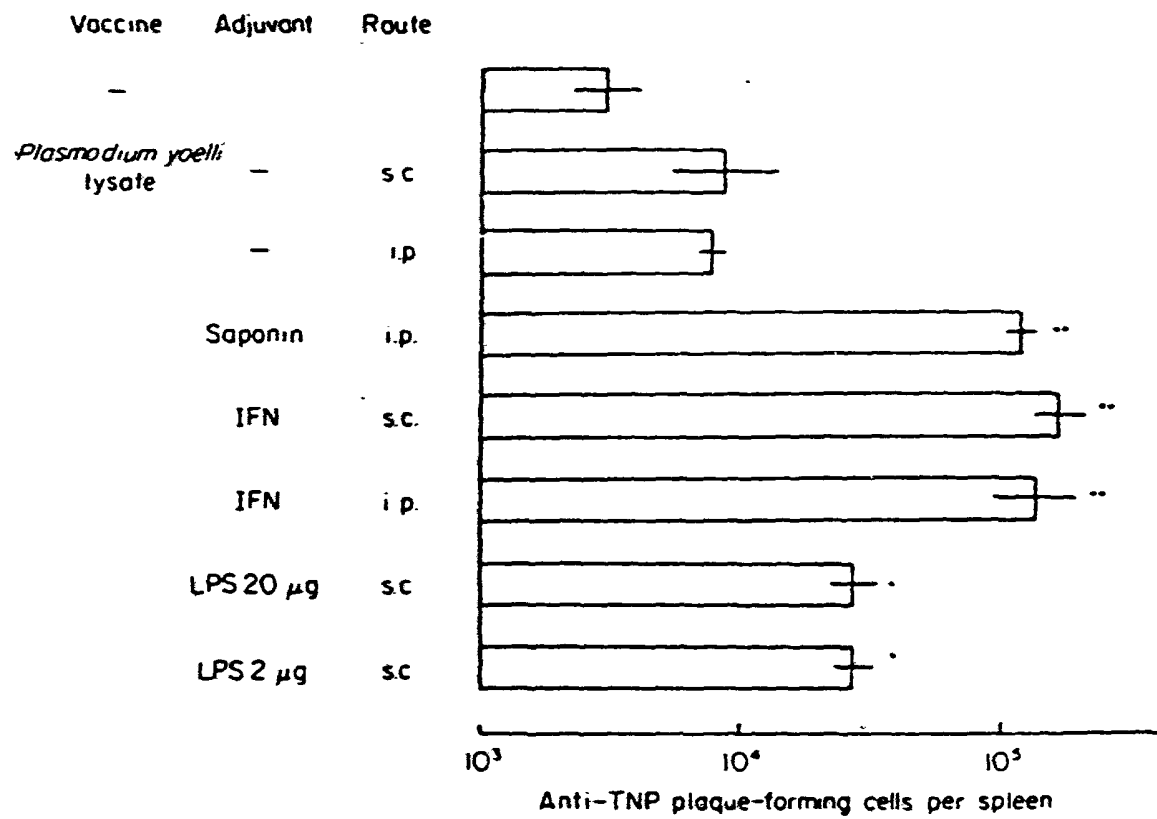


Fig. 1

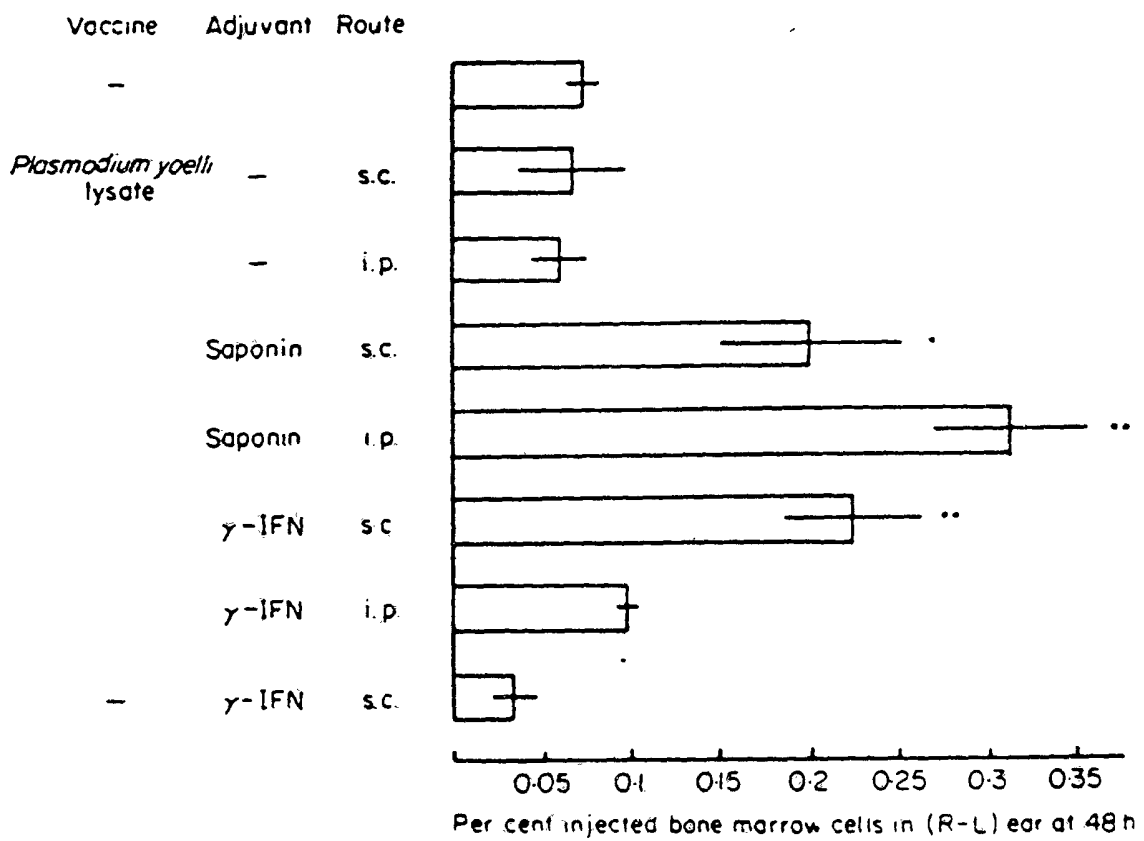


Fig. 2

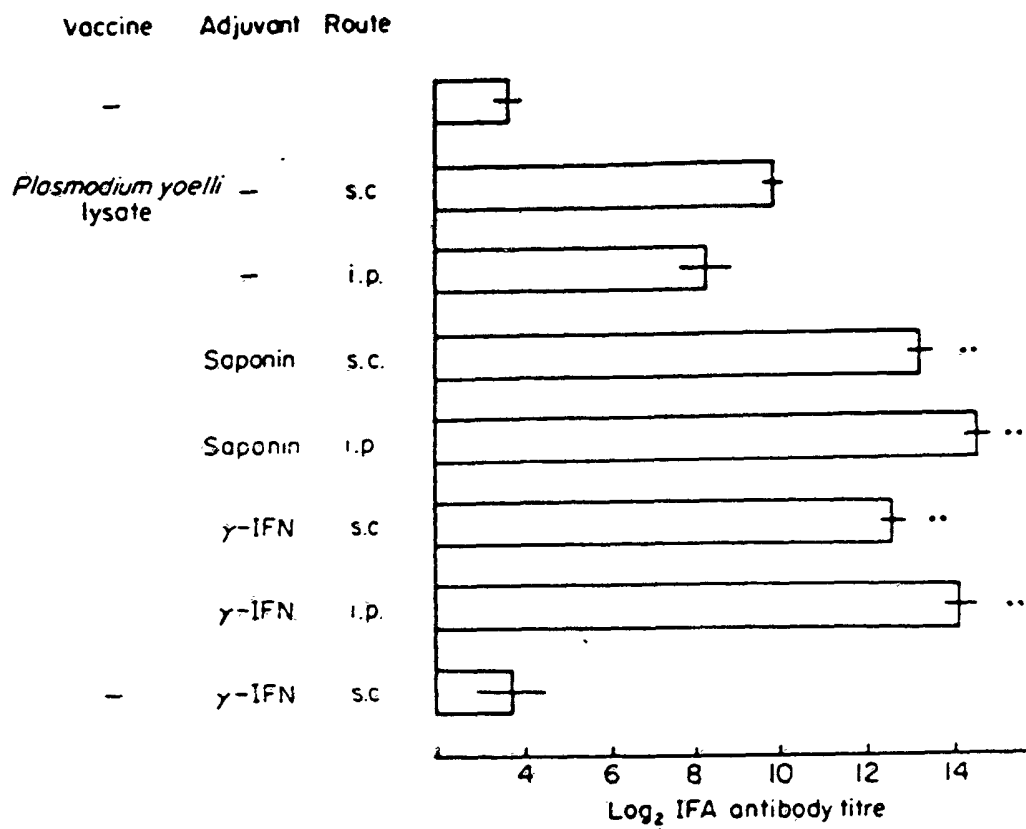


Fig. 3