AUSTRALIA
COMMONWEALTH of AUSTRALIA
PATENTS ACT 1952
APPLICATION FOR A STANDARD PATENT

I, We, BOEHRINGER INGELHEIM INTERNATIONAL GmbH,
Of D-6507 Ingelheim am Rhein,
Federal Republic Of Germany.

hereby apply for the grant of a Standard Patent for an invention entitled:

"MONOCLONAL ANTIBODIES"

which is described in the accompanying provisional specification.

Details of basic application(s):

<table>
<thead>
<tr>
<th>Number</th>
<th>Convention Country</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 36 33 323.9</td>
<td>FEDERAL REPUBLIC OF GERMANY</td>
<td>1st October 1986</td>
</tr>
</tbody>
</table>

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

Dated this 30th day of September 1987

To: THE COMMISSIONER OF PATENTS

(a member of the firm of DAVIES & COLLISON for and on behalf of the Applicant).

Davies & Collison, Melbourne and Canberra.
In support of the Application made for a patent for an invention
entitled: "Monoclonal Antibodies"

I

John Michael Slattery

Of Davies & Collison,

1 Little Collins Street,
Melbourne 3000,
Victoria,
Australia

do solemnly and sincerely declare as follows :-

1. (a) John Michael Slattery

or (b) I am authorized by

Boehringer Ingelheim International GmbH

the applicant............. for the patent to make this declaration on its behalf.

2. (a) Gunther Adolf

or (b) Gunther Adolf

Of Johannagasse 20/7,
A-1050 Wien,
Austria.

The applicant would if a patent were granted on an application made by the said inventor be entitled to have the patent assigned to it.

3. The basic application............. as defined by Section 141 of the Act was made

in Federal Republic of Germany on the 1st October 1986

by Boehringer Ingelheim International GmbH

in on the

by

in on the

by

The basic application............. referred to in paragraph 3 of this Declaration was the first application............. made in a Convention country in respect of the invention the subject of the application.

Declared at Melbourne this 30th day of September 1987

DAVIES & COLLISON, MELBOURNE and CANBERRA.
Hybridoma cells, detection kits are also claimed.

1. A monoclonal antibody of the IgG type or a fragment thereof which is capable of binding omega interferon but does not substantially bind IFN-β, IFN-gamma or α-interferons.

11. A process for preparing a hybridoma cell line as claimed in any of claims 8 to 11 which comprises fusing spleen cells from an animal immunised with IFN-omega or a hybrid interferon, consisting of one part IFN-omega and one part IFN-α, said animal subsequently having been immunised again with IFN-omega, with myeloma cells and subsequently isolating a hybridoma clone which produces a monoclonal antibody as claimed in any of claims 1 to 7.

19. Use of a monoclonal antibody as claimed in any of claims 1 to 7 for purifying an omega interferon.

20. An antibody-affinity carrier wherein a monoclonal antibody as claimed in any of claims 1 to 7 is covalently bound to a suitable carrier material.
28. An immunoassay method for detecting IFN-omega wherein a sample is incubated with an antibody as claimed in claim 5 or claim 6 and the reaction of the antibody with IFN-omega is determined.

29. An immunoassay method for detecting or quantitatively determining IFN-omega wherein a sample

a) is incubated with a carrier to which a monoclonal or polyclonal antibody capable of binding IFN-omega is bound

b) the sample thus obtained is mixed with a monoclonal antibody as claimed in claim 5 or claim 6 and

c) the amount of the monoclonal antibody as claimed in claim 5 or claim 6 thus bound or remaining unbound is determined.
NAME OF APPLICANT: BOEHRINGER INGELHEIM INTERNATIONAL GmbH

ADDRESS OF APPLICANT: D-6507 Ingelheim am Rhein,
Federal Republic of Germany.

NAME(S) OF INVENTOR(S) Gunther ADOLF

ADDRESS FOR SERVICE: DAVIES & COLLISON, Patent Attorneys
1 Little Collins Street, Melbourne, 3000.

COMPLETE SPECIFICATION FOR THE INVENTION ENTITLED:
"MONOCLONAL ANTIBODIES"

The following statement is a full description of this invention, including the best method of performing it known to us
This invention relates to monoclonal antibodies and in particular to monoclonal antibodies against IFN-omega, to processes for preparing them and to their use in the purification and detection of IFN-omega.

New type I interferons, which differed substantially in their structure and antigenic properties from the α- and β-interferons previously known, were described in Nucleic Acids Res. 13, 4739-4749 (1985). This new class of interferon was designated IFN-omega.

Our co-pending European Patent Application No. 236920, which was published on September 16, 1987, describes the improved purification of IFN-omega using antibodies, e.g. monoclonal antibody OMG-2. However, these antibodies show specificity for both IFN-α and IFN-omega and it has not hitherto been possible to set up an immunoassay for detecting IFN-omega using the antibodies described in this application because the proportion of IFN-omega-specific antibodies in the polyclonal coating immunoglobulin was much too small. Moreover, since both the polyclonal and the monoclonal antibodies described also recognise IFN-α such a test would not be specific for IFN-omega.

Thus, prior to the present invention, the detection and quantitative determination of IFN-omega have had to be carried out exclusively by means of biological tests such as, for example, measurement of the antiviral activity. These detection methods are generally very sensitive but are time consuming, laborious and inprecise. It would therefore be highly desirable to provide an immunoassay, for example a test such as an ELISA or IRMA, which could be used to determine IFN-omega simply, quickly and accurately. IFN-omega exists as a monomer in solution...
and thus a test of this kind would require at least two antibodies capable of recognising different epitopes of the IFN molecule; monoclonal antibodies are not essential but have numerous well known advantages when compared to antisera.

Surprisingly, it has now been found that the problems described above can be solved with the aid of the new monoclonal antibodies prepared according to the invention and which are specific for IFN-omega; immunoassays carried out with these antibodies make it possible to detect and quantify IFN-omega without interference from other human interferons such as IFN-α, IFN-β and IFN-gamma.

According to one aspect of the present invention there is provided a monoclonal antibody of the IgG type or a fragment thereof which is capable of binding omega interferon but does not substantially bind IFN-β, IFN-gamma or α-interferons. Preferably the monoclonal antibody wholly or partially neutralises the activity of omega interferon but does not substantially neutralise the activity of IFN-β, IFN-gamma or α-interferons.

In a further aspect of the invention there is provided a hybridoma cell line which produces a monoclonal antibody as hereinbefore defined.

A still further aspect of the invention provides a process for preparing a hybridoma cell line as defined herein which comprises fusing spleen cells from an animal immunised with IFN-omega or a hybrid interferon, consisting of one part IFN-omega and one part IFN-α, said animal subsequently having been immunised again with IFN-omega, with myeloma cells and subsequently isolating a hybridoma clone which produces a monoclonal antibody as hereinbefore defined.

The invention also extends to processes for preparing
the new monoclonal antibodies, to their use for purifying or detecting IFN-omega and to kits containing them.

If an immunogenic substance is introduced into a living host, the immune system of the host normally reacts by forming antibodies to all detectable sites on the immunogenic substance. This effect, namely the formation of antibodies as a defence against an invader, thus results in the production of antibodies of varying affinity and specificity for the immunogenic substance.

Thus, the antibody-producing hybridoma cell lines of the invention may be obtained by cell fusion of spleen cells (see Köhler and Milstein in Nature 256, 495-497 (1975)) from suitably immunised experimental animals, e.g. mouse spleen cells, with myeloma cells, which preferably do not produce any antibodies themselves, e.g. with myeloma cells of the cell line P3X63Ag8.653 (see Kearney et al. in J. Immunol. 123, 1548 (1979)). This process essentially comprises injecting a mouse or other suitable animal with an immunogen, following which the spleen cells thereof are fused with myeloma cells. Hybrid cells are obtained, referred to as hybridoma, which can reproduce in vitro. The hybridoma population is analysed and manipulated to isolate individual clones from each of which a single antigen-specific antibody species is separated. Each individual antibody species obtained in this way is the product of a single B cell from the immunised animal, produced as a reaction to a specific immunogenic structure of the immunogenic substance. Since the preferred two-site immunometric assay is based on the formation of an antibody:antigen:antibody sandwich, two different monoclonal antibodies which do not obstruct each other during bonding to the antigen are generally selected.
In the present case, the experimental animals are previously immunised with an IFN-omega or a hybrid interferon consisting of one part IFN-omega and one part IFN-α, preferably with IFN-omegal or with IFN-omegal/α2, and are subsequently immunised again with an IFN-omega, preferably IFN-omegal.

Following the subsequent cell fusion hybridoma cultures are obtained which are then screened to identify those clones which produce antibodies directed against IFN-omega. Biological tests are preferably used for this, e.g. tests capable of proving that the antibodies produced neutralise IFN-omega biological activity, for example, antiviral activity.

Of five different cultures which were typically obtained, designated OMG-4, OMG-5, OMG-6, OMG-7 and OMG-8, and which consistently showed a reduction in the antiviral activity of IFN-omegal, the clones OMG-4, OMG-5 and OMG-7 were selected for antibody production.

The hybridoma cell lines selected may be cultivated \textit{in vitro} or \textit{in vivo}, but \textit{in vivo} culture is preferred, such as, for example, as described below:

Cells of the selected clones are inoculated into mammals e.g. Balb/c mice which have been pretreated with pristane or incomplete Freund's adjuvant (see for example Müller \textit{et al.} in J. Immunol. Methods 87, 193-196 (1986)). After 7-18 days the ascitic fluid is collected and the antibody formed is concentrated or isolated from it, for example, by precipitation with ammonium sulphate and subsequent affinity chromatography or by other methods known from the literature.
Naturally, the desired antibody may be isolated or concentrated analogously from a cell culture supernatant of a suitable in vitro culture.

5 As already mentioned hereinbefore, a new antibody thus prepared according to the invention may be used for the purification and detection of IFN-omega, preferably IFN-omegal.

10 If the antibody obtained according to the invention is to be used for the ultra-purification of an IFN-omega, it is preferably covalently bonded to a biologically inactive carrier. The antibody may be covalently bonded to a suitably activated carrier, preferably dextran-based, e.g. CNBr-activated Sepharose or activated CH-Sepharose made by Messrs. Pharmacia of Uppsala. For ultra-purification, a solution of the omegal interferon which is to be purified, and which is conveniently obtained either by the processes described in EP-A-0170204 or by means of the new plasmids described in EP-A-0236920, may be pumped over an antibody affinity carrier thus prepared at a slightly basic pH, e.g. at pH 7-8, but preferably at pH 7.5, then washed at pH 7.5 until the eluate is free from protein, and subsequently the bound interferon may be eluted in the acidic range, e.g. using 0.1 molar citric acid in 25% ethylene glycol. The protein-containing fractions thus obtained may be subsequently chromatographed over a strongly acidic cation exchanger, e.g. the cation exchanger Mono-S made by Pharmacia. The human interferon from the above eluate is immediately absorbed by the cation exchanger column and may subsequently be eluted by means of, for example, an NaCl gradient.

If the new antibodies are to be used in the detection
or quantitative determination of an omega interferon, e.g. IFN-omegal as antigen, the conventional immunoassay techniques may be used.

5 These techniques are based on the formation of a complex between the "antigenic" substance to be determined and one or more antibodies, in which one or several parts of the complex may be labelled, so that after the complexed "antigen" or antibody has been separated off it is possible to detect and/or quantitatively determine the "antigen".

In the case of a competitive immunoassay technique the "antigenic" substance in a liquid sample which is to be investigated is in competition with a known quantity of a labelled "antigen" for a limited quantity of antibody binding sites. Therefore, the quantity of labelled "antigen" bound to the antibody is in inverse proportion to the quantity of "antigen" in the sample.

Immunometric methods, on the other hand, use labelled antibodies. In an assay of this kind, the quantity of labelled antibody bound to the complex is proportional to the quantity of "antigenic" substance contained in the liquid sample.

Immunometric assays are particularly suitable for the detection of polyvalent "antigens", i.e. "antigenic" substances which are capable of forming a complex with two or more antibodies simultaneously. Assays of this kind typically use a quantity of an unlabelled antibody, bound to a solid carrier, which is insoluble in the liquid which is to be investigated, and a quantity of a soluble antibody which is labelled, so that it is possible to detect and/or quantitatively determine the quantity of the ternary complex which
forms between the solid phase antibody, the "antigen" and the labelled antibody.

To do this, normally the antibody bound to the solid phase is first brought into contact with the sample being investigated in order to extract the "antigen" from the sample by forming a binary solid phase-antibody:antigen complex. After a suitable incubation period the solid carrier is washed in order to remove the residues of the liquid sample, including any unreacted "antigen" which may be present, and is then contacted with a solution which contains a known quantity of the labelled antibody.

After a second incubation period in which the labelled antibody is allowed to form a complex with the "antigen", which is bound to the solid carrier by the unlabelled antibody, the solid carrier is washed a second time in order to remove any unreacted labelled antibody. In a simple "Yes/No" assay to determine whether there is any antigen in the sample under investigation, the washed solid carrier is investigated. The quantity of labelled antibody detected is compared with that of a negative control sample which is free from the "antigen". The detection of labelled antibody in quantities considerably higher than the background level, which would be indicated by a negative control, then indicates the presence of the suspected antigen. Quantitative detection is possible by comparing the measurements of labelled antibodies which are obtained with calibrated samples containing known quantities of the "antigen". This type of assay is frequently referred to as a "two-site" or "sandwich" assay since the antigen has two antibodies bound to different sites on its surface.
In the assays described hereinbefore, for example

the carrier may be a conventional carrier such as glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylase, natural or chemically modified cellulose, polyacrylamide, agarose or magnetite and

the markers may be enzymes, radioisotopes, metal chelates or fluorescent, chemiluminescent and bioluminescent compounds.

Examples of enzymes include malate dehydrogenase, Staphylococcal nuclease, delta-5-steroid isomerase, α-glycerol phosphate dehydrogenase, triosephosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase or acetylcholinesterase;

the radioisotope used may be \( ^{3}\text{H} \), \( ^{125}\text{I} \), \( ^{127}\text{I} \), \( ^{32}\text{P} \), \( ^{35}\text{S} \) or \( ^{14}\text{C} \);

the fluorescent compounds may be fluorescein isothiocyanate, rhodamine, phycoerythrin, phycoerythrin, allophycocyanin, o-phthaldehyde or fluorescamine;

the chemiluminescent compounds may be luminol, isoluminol, an aromatic acridinium ester, imidazole, an acridinium salt or an oxalic acid ester; and

the bioluminescent compounds may be luciferin, luciferase or aequorin.

Moreover, an antibody according to the invention may be linked to a low molecular weight hapten such as biotin, dinitrophenyl, pyridoxal or fluorescamine.
These haptens may then be recognised by a further specific reaction, e.g. biotin with the aid of avidin or fluorescamine with the aid of a specific antihapten antibody.

Moreover, the activity of an enzyme used as a marker may be used to intensify the signal which is being measured.

However, it is particularly preferred to use horseradish peroxidase as the marker since this enzyme is capable of reacting with numerous substrates. Moreover, it is relatively small and can easily be linked to an antibody, for example by the periodate method.

However, the preferred methods of detecting or quantitatively determining an omega interferon, preferably IFN-omegal, are, if the IFN-omega is radioactively labelled, competitive radioimmunoassay (RIA) in which polyclonal antibodies or antibody sera are used; in particular, immunoradiometric assay (IRMA) if the antibody is radioactively labelled, and "enzyme-linked immunosorbent assay" (ELISA), if the antibody is labelled with an enzyme.

According to the invention, IFN-omega, but preferably IFN-omegal, may be detected or quantitatively determined in a test liquid as follows:

a) by contacting the sample which is to be investigated with a carrier to which a polyclonal or monoclonal antibody against the IFN-omega which is to be determined is bound, and

b) measuring the formation of the binary complex formed under a), by formation of a ternary complex
between a labelled monoclonal antibody and the binary complex formed according to a).

The omega interferons needed to carry out this invention are the subject of EP-A-0170204 and the monoclonal antibodies, e.g. of antibody OMG-2, which are not described in the present invention, are the subject of EP-A-0236920; the same applies to the hybrid interferons used for immunisation.

The polyclonal antibodies used are obtained using methods known from the literature.

The following Examples are intended to illustrate the present invention more fully:
Example 1

Preparation of monoclonal antibodies specific to IFN-omega

a) Immunisation

A female Balb/c mouse about 8 weeks old was immunised with highly purified (purity > 95%) hybrid interferon IFN-omega as follows:

1st immunisation: 200 mcg in complete Freund's adjuvant, by intraperitoneal route

2nd immunisation: 200 mcg in complete Freund's adjuvant, by intraperitoneal route, 5 weeks after the 1st immunisation

Eight months after the second immunisation the mouse was immunised again with 70 mcg of purified IFN-omega (purity > 90%) (incomplete adjuvant, intraperitoneal route). Twelve days later a serum sample was taken. Neutralisation tests showed that the mouse's serum now contained relatively high titres of neutralising antibodies against IFN-omega (total neutralisation at up to 1000-fold dilution of the serum, partial neutralisation at 10,000 fold dilution). The neutralisation test was carried out as follows: 100 mc1 of a dilution of the serum sample in cell culture medium were mixed with 100 mc1 of a solution of IFN-omega (100 antiviral units/ml) and incubated for 90 minutes at 37°C. The antiviral activity of the samples was then tested in a biological test (A549 lung cancer cells, encephalomyocarditis virus). Five
weeks after the third immunisation, the mouse was injected with a further 70 mcg of purified IFN-omegal (purity > 90%) without any adjuvant.

b) Production and screening of hybridomas

Hybridomas were produced using the method originally developed by Köhler and Milstein (Nature 256, 495 (1975)) using the non-secreting cell line P3X63Ag8.653 (Kearney et al., J. Immunol. 123, 1548 (1979)). The following procedure was used:

Four days after the last immunisation (see above) the mouse's spleen was removed; the spleen cells were mechanically freed from the connecting tissue, suspended in cell culture medium (RPMI 1640 medium with added sodium penicillin G (100 units/ml) and streptomycin sulphate (50 units/ml)) and collected by centrifuging (Beckmann TJ-6 centrifuge, 10 minutes at 1000 rpm). 2x10^8 myeloma cells (cultivated in cell culture medium as above with the addition of 10% foetal calf serum) were also collected by centrifuging and washed once with serum-free cell culture medium. Finally, the spleen cells and myeloma cells were resuspended in serum-free cell culture medium, the suspensions were combined and centrifuged again. The supernatant was removed, the cells were suspended in 3 ml of fusion medium (45% RPMI 1640 medium, 50% polyethylene glycol 4000, 5% dimethylsulphoxide) and carefully shaken for 90 seconds, then left to stand for a further 60 seconds. 3 ml of serum free culture medium were then added dropwise over a period of 90 seconds, the suspension was left to stand for 60 seconds, then a further 6 ml of serum free culture medium were added dropwise over a period of 90 seconds. Finally, 12 ml of culture medium containing 10%
foetal calf serum were slowly added with constant stirring, left to stand for 10 minutes and then the mixture was made up to 50 ml with cell culture medium containing 10% foetal calf serum. The cells were collected by centrifuging and suspended in 400 ml of cell culture medium with the addition of 20% foetal calf serum and hypoxanthine (10^{-4} M), aminopterin (4x10^{-7} M) and thymidine (1.6x10^{-5} M), hereinafter referred to as HAT medium. Furthermore, peritoneal macrophages from Balb/c mice were also added to this suspension (about 50,000/ml); the suspension was finally pipetted into cell culture plates (48 wells per plate; 0.5 ml per well). The plates were incubated at 37°C (95% air, 5% CO_{2}, saturated water vapour). After 3 days 0.5 ml of HAT medium were added to each culture. Of the total of 800 cultures set up, about 300 cultures showed some growth of hybridoma cells after two to three weeks. The subsequent screening was carried out as follows:

Culture supernatants of at least 10-20% confluent hybridoma cultures were mixed with equal volumes of a solution of HuIFN-omegal (20 antiviral units/ml), incubated for 90 minutes at 37°C and then tested for their antiviral activity. All cultures were tested at least twice at intervals of one week. Five of the cultures, hereinafter designated OMG-4, OMG-5, OMG-6, OMG-7 and OMG-8, consistently showed a reduction in antiviral activity in all the tests. All the cultures were cloned by limiting dilution, the clones were tested again for any neutralising activity using the method described. From each culture, 3 to 5 positive clones were pooled. In order to produce antibodies in vivo, from each of the hybridoma cultures 3-10x10^6 cells were inoculated by intraperitoneal route into Balb/c mice which
had been intraperitoneally injected, two to three days previously, with 0.5 ml of incomplete Freund's adjuvant or with 0.5 ml of pristane seven to ten days previously. After seven to 21 days the ascitic fluid formed was recovered; the antibodies contained in it were concentrated to a purity of over 90% by precipitation with 50% ammonium sulphate and affinity chromatography over carrier-bound protein A by known methods. For each ml of ascitic fluid about 2-5 mg of pure antibodies were obtained from all the hybridomas.

c) Characterisation of the antibodies OMG-4, OMG-5 and OMG-7

When examined by sodium dodecylsulphate-polyacrylamide electrophoresis under non-reducing conditions and gel permeation high pressure liquid chromatography, all the antibodies showed retention characteristics identical to those of an IgG marker protein and are therefore of the IgG type. In a neutralisation test in which the inhibition of antiviral activity of interferons was investigated (see above), all the antibodies neutralised the activity of IFN-omegal at a concentration of 100 mcg/ml but did not neutralise the activity of IFN-α2c, IFN-β or IFN-gamma.

These three clones were deposited according to the Budapest Treaty at the European Collection of Animal Cell Cultures, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom, on 14 August 1987, under the ECACC-file numbers 87 081401 (OMG-4), 87 081402 (OMG-5) and 87 081403 (OMG-7).
Example 2

Enzyme immunoassays (ELISA) for IFN-omegal

The antibodies OMG-5 and OMG-7 were covalently bound to horseradish peroxidase using known methods (see for example Wilson M.B. and Nakane P.K., in Immunofluorescence and Related Staining Techniques, published by W. Knapp et al., pages 215-224; Elsevier 1978). The procedure used was as follows:

2 mg of horseradish peroxidase in water were mixed with 0.2 ml of 100 mM sodium periodate and shaken for 40 minutes at ambient temperature, then dialysed against 2 x 500 ml of 1 mM sodium acetate, pH 4.4, overnight at 4°C; the solution was then adjusted to a pH of about 9 using 0.1 M NaHCO₃, pH 9.5. A solution of the monoclonal antibody (OMG-5, 2 ml with 1.6 mg/ml or OMG-7; 1.5 ml with 4.7 mg/ml, each in 10 mM NaHCO₃, pH 9.5) was added to this solution and the resulting mixture was shaken for 2 hours at ambient temperature. 100 ml of a solution of NaBH₄ (4 mg/ml in water) were added and the solution was incubated for a further 2 hours in an ice bath; then 3 ml of cold saturated ammonium sulphate solution were added dropwise and the mixture was incubated for 1 hour in an ice bath. The precipitate of peroxidase-immunoglobulin conjugate formed was collected by centrifuging, dissolved in 1 ml of phosphate-buffered isotonic saline solution pH 7.4 and stabilised by the addition of 1 ml of a solution of bovine serum albumin (10 mg/ml) in phosphate-buffered saline solution. The solution was frozen at -70°C.

Solid phase sandwich enzyme immunoassays for IFN-omegal were carried out using generally known methods.
In order to coat the microtitre F.I.S.A test plates the monoclonal antibodies OMG-2 OMG-5 or OMG-7 were used in a concentration of 10 mcg/ml in 0.1 M sodium carbonate pH 9.5 (100 mcl per well) and the plates were incubated either for 1 hour at ambient temperature or overnight at 4-8°C. The antibody solution was removed, the wells were each washed with 200 mcl of water and filled with 100 mcl of a solution of bovine serum albumin (5 mg/ml) in phosphate-buffered isotonic saline solution pH 7.4 (hereinafter referred to as PBS/BSA). Then 100 mcl of a solution of IFN-omegal in a concentration of 20 ng/ml were added, mixed in and a series of dilutions was produced by serial transfer of 100 mcl. Finally, 50 mcl of a solution of the antibody-enzyme conjugate (OMG-5/peroxidase or OMG-7/peroxidase, original solution (see above) 1:10,000 dilution in PBS/BSA) were added to all the wells and the plates were incubated for 3 hours at ambient temperature. Then the solution was removed, the wells were washed three times with water and each filled with 100 mcl of substrate solution (ortho-phenylenediamine, 3 mg/ml and sodium perborate, 1 mg/ml in 0.067 M potassium citrate pH 5). After 30 minutes' incubation at ambient temperature 100 mcl of 4 N sulphuric acid were pipetted into each well; then the optical density at 492 nm was measured in a multi-channel photometer (ELISA reading apparatus).

Dosage-dependent changes in absorption were achieved with all the heterologous combinations of coating antibodies and antibody-peroxidase conjugate. Figures 1, 2 and 3 show the curves obtained.

Coating may also be carried out using a rabbit
anti-IFN-omega immunoglobulin obtained by twice immunising a rabbit with IFN-omega and partial purification from the serum by precipitation with 50% ammonium sulphate in a concentration of 10 mcg/ml (see Figure 4).

The specificity of the IFN-omega ELISA (see Figure 2) constructed from the antibody OMG-2 (see EP-A-0236920) and peroxidase-bound antibody OMG-7 was tested by applying preparations of other human interferons over a very wide range of concentrations. The following interferons were used:

<table>
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<tr>
<th>Interferon</th>
<th>Source</th>
<th>Concentration range</th>
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<tr>
<td>IFN-α1</td>
<td>recombinant (E. coli)</td>
<td>2 ng - 50 mcg/ml</td>
</tr>
<tr>
<td>IFN-α2c</td>
<td>recombinant (E. coli)</td>
<td>2 ng - 50 mcg/ml</td>
</tr>
<tr>
<td>IFN-αβ</td>
<td>recombinant (E. coli)</td>
<td>3x10^2-1.25x10^6 U/ml</td>
</tr>
<tr>
<td>IFN-αF</td>
<td>recombinant (E. coli)</td>
<td>1.4x10^1-3.5x10^5 U/ml</td>
</tr>
<tr>
<td>IFN-β</td>
<td>fibroblasts, induced with Poly (I:C)</td>
<td>8x10^2-2x10^6 U/ml</td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>recombinant (E. coli)</td>
<td>2 ng - 50 mcg/ml</td>
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At a sensitivity of 100 pg/ml for IFN-omega no significant signal was observed with any of the preparations at any concentration. The ELISA can therefore be used not only to quantify recombinant IFN-omega but also, for example, to determine the proportion of IFN-omega in leucocyte interferon or other interferon preparations obtained from cell cultures.
Example 3

Immunoradiometric assay (IRMA) for IFN-omegal

The monoclonal antibody OMG-7 was radioactively labelled with N-succinimidyl[2,3-\(^3\)H]propionate (\(^3\)H-NSP, made by Amersham International, England; 110 Ci/mmol) using a known method. 1 mCi of the solution of \(^3\)H-NSP was brought to dryness in vacuo in a siliconised test vessel. Then 50 mcg of a solution of the monoclonal antibody OMG-7 (4.7 mg/ml) in buffered saline solution pH 7.4 were pipetted in and 3 mcl of 1 M borate buffer pH 8.5 were added. After 24 hours at 4°C the excess \(^3\)H-NSP was captured with 20 mcl of 1 M glycine in borate buffer, diluted with 250 mcl of 50 mM potassium phosphate buffer pH 7.4 containing 150 mM NaCl and 5 mg/ml of bovine serum albumin and separated from the labelled antibody over a Sephadex G 50 M column (0.5 x 20 cm). The antibody showed a specific activity of about 10 Ci/g of protein.

In order to carry out the test, etched polystyrene pellets (diameter 6.5 mm; made by Northumbria Biologicals of England) were coated with the antibody OMG-2 (10 mcg/ml in 0.1 M sodium carbonate pH 9.5; 1 hour at ambient temperature). The pellets were then incubated for 1 hour in PBS/BSA (see Example 2) and then washed twice with 250 mcl of water. The pellets were incubated in suitable test tubes with 200 mcl of a solution of IFN-omegal in increasing concentrations in PBS/BSA for 3 hours at 4°C and washed three times with 250 mcl of water. Then 200 mcl batches of a solution of the labelled antibody (100 ng/ml in PBS/BSA; about 27,000 counts per minute per test tube) were added and the resulting mixture was incubated for 20 hours at 4°C. The
pellets were then washed three times with 20 μl of water, transferred into polypropylene tubes and the bound radioactivity was measured in a liquid scintillation counter after the addition of 4 ml of scintillation cocktail. Figure 5 shows the bound radioactivity as a function of the interferon concentration.
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A monoclonal antibody of the IgG type or a fragment thereof which is capable of binding omega interferon but does not substantially bind IFN-β, IFN-gamma or α-interferons.

2. A monoclonal antibody as claimed in claim 1 which wholly or partially neutralises the activity of omega interferon but does not substantially neutralise the activity of IFN-β, IFN-gamma or α-interferons.

3. A monoclonal antibody as claimed in claim 2 which neutralises only the activity of IFN-omega.

4. A monoclonal antibody as claimed in claim 2 or claim 3 which, at a concentration of 100 mcg/ml, neutralises the activity of IFN-omega but does not neutralise the activity of IFN-α2c, IFN-β or IFN-gamma.

5. A monoclonal antibody as claimed in any of claims 1 to 4 which is labelled.

6. A monoclonal antibody as claimed in claim 5 wherein the labelling is effected using a radioactive isotope, enzyme, fluorescent compound, chemiluminescent compound or a bioluminescent compound.

7. A monoclonal antibody as claimed in claim 1 substantially as described herein.

8. A hybridoma cell line which produces a monoclonal antibody as claimed in any of claims 1 to 7.

9. A hybridoma cell line as claimed in claim 8 selected from the hybridoma designated OMG-4.
A hybridoma cell line as claimed in claim 8 substantially as described herein.

11. A process for preparing a hybridoma cell line as claimed in any of claims 8 to 11 which comprises fusing spleen cells from an animal immunised with IFN-omega or a hybrid interferon, consisting of one part IFN-omega and one part IFN-α, said animal subsequently having been immunised again with IFN-omega, with myeloma cells and subsequently isolating a hybridoma clone which produces a monoclonal antibody as claimed in any of claims 1 to 7.

12. A process as claimed in claim 11 wherein IFN-omega or IFN-omega/α2 is used for the first immunisation.

13. A process as claimed in claim 11 or claim 12 wherein IFN-omega is used for the second immunisation.

14. A process as claimed in any of claims 11 to 13 wherein the spleen cells from Balb/c mice are used.

15. A process as claimed in any of claims 11 to 14 wherein myeloma cells of the cell line P3X63Ag8.653 are used.

16. A process as claimed in claim 11 substantially as described herein.

17. A monoclonal antibody as claimed in any of
claims 1 to 7 prepared by cultivating in vitro or in vivo a hybridoma cell line as claimed in any of claims 8 to 10.

5 18. A process for the preparation of a monoclonal antibody as claimed in any of claims 1 to 7 which comprises culturing a hybridoma cell line producing said antibody, isolating said antibody from the culture media and, if desired, subsequently labelling the antibody thus isolated.

19. Use of a monoclonal antibody as claimed in any of claims 1 to 7 for purifying an omega interferon.

15 20. An antibody-affinity carrier wherein a monoclonal antibody as claimed in any of claims 1 to 7 is covalently bound to a suitable carrier material.

21. Process for preparing an antibody-affinity carrier as claimed in claim 20 wherein a monoclonal antibody as claimed in any of claims 1 to 7 is covalently bound to a suitable carrier material.

22. A process for purifying an omega interferon wherein said IFN-omega is bound to an antibody-affinity carrier as claimed in claim 20 and is subsequently eluted therefrom.

23. Use of a monoclonal antibody as claimed in any of claims 1 to 7 for detecting IFN-omega.

24. A kit suitable for detecting IFN-omega comprising a monoclonal antibody as claimed in claim 5 or claim 6.

25. A kit suitable for detecting or quantitatively determining IFN-omega comprising
a) a solid phase to which a monoclonal or polyclonal antibody capable of binding IFN-omega is bound and

b) a monoclonal antibody as claimed in claim 5 or claim 6.


27. A kit as claimed in claim 24 substantially as described herein.

28. An immunoassay method for detecting IFN-omega wherein a sample is incubated with an antibody as claimed in claim 5 or claim 6 and the reaction of the antibody with IFN-omega is determined.

29. An immunoassay method for detecting or quantitatively determining IFN-omega wherein a sample

a) is incubated with a carrier to which a monoclonal or polyclonal antibody capable of binding IFN-omega is bound

b) the sample thus obtained is mixed with a monoclonal antibody as claimed in claim 5 or claim 6 and

c) the amount of the monoclonal antibody as claimed in claim 5 or claim 6 thus bound or remaining unbound is determined.

30. An immunoassay as claimed in claim 28 or claim 29 substantially as described herein.
31. Each and every novel antibody, hybridoma, use, process and kit herein disclosed.

Dated this 30th day of September 1987.

BOEHRINGER INGELHEIM INTERNATIONAL GmbH
By its Patent Attorneys
DAVIES & COLLISON
DRAWINGS
FIG. 1.

ELISA for IFN-omega 1

Coating: Monoclonal antibody OMG-2
Peroxidase Conjugate: Monoclonal antibody OMG-5
FIG. 2.
ELISA for IFN-omega 1
Coating: Monoclonal antibody OMG-2
Peroxidase Conjugate: Monoclonal antibody OMG-7

![Graph showing optical density (492 nm) vs. ng/ml]
FIG. 3.

ELISA for IFN-omega1

Coating: Monoclonal antibody OMG-5
Peroxidase Conjugate: Monoclonal antibody OMG-7

Optical Density (492 nm)

ng/ml
FIG. 4.
ELISA for IFN-omega 1
Coating: Rabbit anti-IFN-omega 1 immunoglobulin
Peroxidase Conjugate: Monoclonal antibody OMG-7

![Graph showing optical density against ng/ml]
FIG. 5.

Immunoradiometric assay for HuIFN-omega 1

Counts/min

ng/ml

0 1 3 10 30 100