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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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Solicitud de Patente

En relación con la última providencia recaída en este expediente, me permito manifestarle lo siguiente:

- 1.- Debidamente traducida, acompaño la copia oficial de la solicitud de patente inglesa No. 9901254.4.
- 2.- Modifico el título de la invención en la siguiente forma

"VACUNAS QUE COMPRENDEN PROTEINAS DE FUSION DEL VIRUS VARICELA ZOSTER"
- 3.- Las secuencias que el Señor Examinador hecha de menos, se encuentran dentro del texto de la descripción, así:
 - a.- SEC ID No. 1 : Renglón 8 de la Página 15 /
 - b.- SEC ID No. 2 : Renglones 17 a 20 de la Página 16 /
 - c.- SEC ID No. 3 : Renglones 10 y 11 de la Página 18

- 4.- De acuerdo a lo anterior, acompaño nueva carátula, extracto, tarjeta de archivo temático y tarjeta de propietario.

Renuncio al término faltante de traslado y les solicito ordenar la publicación del extracto.

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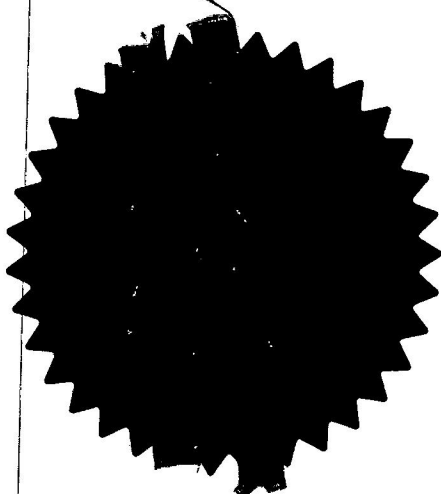
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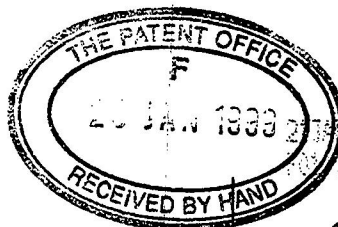


Signed *Andrew Gersey*

Dated 22 December 1999

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9901254.4



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Your Reference: FB/B45165

20 JAN 1999

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1 Please give the title of the invention
VACCINES

② Applicant's details

☐ First or only applicant

2a If you are applying as a corporate body please give:
Corporate Name SmithKline Beecham Biologicals s.a.

Country (and State of incorporation, if appropriate)
Belgium

2b If you are applying as an individual or one of a partnership please give in full:

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Forenames

2c In all cases, please give the following details:

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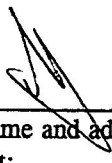
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4. Agent's or
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5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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No ☒ ➔ go to 6



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Claim(s) 3 Abstract —	Description 18 Drawing(s) 12		
8b Which of the following documents also accompanies the application? Priority documents (please state how many) — Translation(s) of Priority documents (please state how many) — Patents Form 7/77 - Statement of Inventorship and Right to Grant — Patents Form 9/77 - Preliminary Examination Report — Patents Form 10/77 - Request for Substantive Examination —			
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Please return the completed form, attachments and duplicates where requested, together with the prescribed fee to either; <table border="0"> <tr> <td> ☐ The Comptroller or The Patent Office Cardiff Road Newport Gwent NP9 1RH </td> <td> ☒ The Comptroller The Patent Office 25 Southampton Buildings London WC2A 1AY </td> </tr> </table>		☐ The Comptroller or The Patent Office Cardiff Road Newport Gwent NP9 1RH	☒ The Comptroller The Patent Office 25 Southampton Buildings London WC2A 1AY
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VACCINES

The present invention relates to compositions for the treatment or prevention of Zoster in individuals infected with Varicella Zoster virus (VZV), and to the prevention or treatment of Varicella infections.

Varicella Zoster Virus is a human alpha herpes virus which causes two human diseases: on primary infection VZV causes childhood chicken pox (Varicella) thereafter the virus becomes latent and frequently reactivates (often decades later) to produce shingles (Zoster). During chicken-pox, the virus penetrates the peripheral nervous system where it remains latent until reactivates as the painful Zoster form. Whilst the virus is latent the expression of most viral genes are repressed. It is believed that cell mediated immunity plays a crucial role in the control of latency, since reactivation as Zoster (or shingles) is frequent in the elderly or in immunocompromised individuals.

VZV infection is characterized by minimal presence of free virus. During latency and reactivation virus is mainly intracellular. Accordingly, recurrent disease is not prevented even by high levels of neutralizing antibodies and virus control depends on cell mediated immunity. In order to obtain protection by vaccination, it is therefore desirable to induce not just an antibody response, but also a Cytotoxic T lymphocytes (CTL) response. An effective vaccine should prime CTL capable of acting as early as possible as soon as signs of reactivation of latent virus appear.

As the mechanism of antigen recognition by Cytotoxic T lymphocytes (CTL) involves breakdown of native antigen into peptides, binding of the proteolytic fragments to MHC molecules and export of the complex to the cell surface, any virus coded polypeptide not just those that are integral membrane proteins like the glycoproteins, can be a potential target of a T cell mediated response. However since the VZV genome codes for several non structural proteins and internal virion proteins, in addition to external glycoproteins, this results in a large number of potential CTL targets and it is not known which protein would be the most relevant.

The genome of VZ Virus is composed of 71 open reading frames, encoding 68 proteins. The sequence of the virus DNA is known (Davison and Scott 1986). The Varicella Zoster Virus protein gE is encoded by the open reading frame 68 and is the most abundant and immunogenic protein amongst the 6 Varicella-Zoster Virus envelope glycoproteins. It is predominant in VZV-infected human cell membranes (Keller et al 1984; Montalvo and Grose 1986; Dubey et al 1988). Anti-gE antibodies have been detected in subjects with natural primary infection and in subjects vaccinated with the live OKA-VZV vaccine. It has been demonstrated that gE is more immunogenic than gB and gH, the two other predominant glycoproteins of VZV (Brunell et al 1987). A monoclonal antibody raised to gE is capable of neutralising infectious virus in vitro (Grose 1989).

The present invention provides a fusion protein comprising

- (1) a Varicella Zoster Virus gE protein or an immunologically active fragment thereof fused to
- (2) a different protein of Varicella Zoster Virus or an immunologically active fragment thereof.

Different proteins of Varicella Zoster Virus include any other structural and non structural proteins from VZV, other immediate early or early proteins or proteins such as gB, gH, gC or gI.

In a further aspect of the invention there is provided recombinant DNA or RNA encoding the fusion protein of the invention. The recombinant DNA or RNA of the invention may form part of a vector, for example a plasmid, especially an expression plasmid from which the fusion protein may be expressed, or a recombinant live microorganism, such as a virus or bacterium. Such vectors also form part of the invention, as do host cells into which the vectors have been introduced.

In yet another aspect of the invention there is provided a vaccine composition comprising a fusion protein according to the invention, or comprising a nucleic acid encoding the fusion protein of the invention in combination with a pharmaceutically acceptable excipient.

In a preferred aspect the fusion protein of the invention comprises as component (2) the VZV immediate early protein IE 63 or an immunologically active fragment thereof.

The Varicella Zoster Virus immediate early protein IE 63 (also called iep34) is encoded by open reading frame 63. It has a predicted molecular mass of 30.5kDa (Debrus et al, J. of Virology 69(5), 1995 p 3240) and is expressed in the early phases of the VZV infectious cycle. The gene starts at 110581 (START CODON) and goes through to 111414 (STOP CODON). Another copy of the gene in reverse orientation is found between base 119316 to 118483. The role of IE63 (also termed ie63) in the cell-mediated immune response has been demonstrated. Rather unusually the IE 63 protein has been shown to be expressed in a rat model, in neurons during latency. The protein has been expressed as a fusion protein in E.coli (Debrus et al).

It has now been discovered that VZV IE 63 protein is detected exclusively in the cytoplasm of neurons of latently infected human trigeminal and thoracic ganglia. This is the first identification of an alpha-herpesvirus protein expressed during latency in the human nervous system. The VZV IE63 protein is an important target for the immune system, and in particular is a T cell response target and thus is useful in the prevention and treatment of VZV infections, in particular, in the prevention of Zoster in patients already infected with VZV.

It has now been surprisingly found that a vaccine composition comprising a fusion protein of VZV gE and VZV IE63 stimulate both humoral and cell-mediated responses against both gE and IE63. The level and quality of these responses is similar to that observed by immunisation with individual gE and IE63 proteins but the fusion protein gE-IE63 can be purified more easily than the proteins alone and thus shows an obvious industrial advantage.

Accordingly, the present invention preferably provides a fusion protein comprising

- (1) a Varicella Zoster Virus gE protein or an immunologically active fragment thereof fused to
- (2) a Varicella Zoster Virus IE63 protein or an immunologically active fragment thereof.

This is the first medical use for such a protein and thus the invention accordingly provides the fusion protein combining VZV gE and a structural or non-structural protein of VZV, in particular VZV IE63 for use in medicine.

In order to construct the DNA encoding a fusion protein according to the invention, cDNA containing the coding sequences of the proteins to be fused may be manipulated using standard techniques [see for example Maniatis T. et al Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y. (1982)].

In a further aspect of the invention there is provided a method of treating a human susceptible to or suffering from VZV infection, which comprises administering to a human, a safe and effective amount of a vaccine composition according to the invention.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccines. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 µg of protein, preferably 2-100 µg, most preferably 4-40 µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

In addition to vaccination of persons susceptible to VZV infections, the vaccine compositions of the present invention may be used to treat, immunotherapeutically, patients suffering from VZV infections, in order to prevent or significantly decrease recurrent disease, frequency, severity or duration of shingles episodes.

In a preferred vaccine of the invention, an aqueous solution of the fusion protein combining VZV gene gE and VZV gene IE63, can be used directly. Alternatively, the fusion protein, with or without prior lyophilization, can be mixed together or with any of the various known adjuvants. Such adjuvants include, but are not limited to, aluminium hydroxide, muramyl dipeptide and saponins such as Quil A, in particular QS21 or 3 Deacylated monophosphoryl

lipid A (3D-MPL), and CpG oligonucleotides (see University of Iowa; WO9602555). Adjuvants or adjuvant systems that preferentially induce a TH1 response are preferred. Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application Nos. WO 94/00153 and WO 95/17209.

A particular preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina, and 3 De-O-acylated monophosphoryl lipid A (3 D-MPL), optionally together with an oil in water emulsion.

3 De-O-acylated monophosphoryl lipid A is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International Patent Application No. 92/16556.

QS21 is a Hplc purified non toxic fraction of a saponin from the bark of the South American tree Quillaja Saponaria Molina and its method of its production is disclosed (as QA21) in US patent No. 5,057,540.

A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and tween 80. Additionally the oil in water emulsion may contain span 85 and/or lecithin.

The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1 : 5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5:1 to 1:1 3D MPL: QS21. Typically for human administration QS21 and 3D MPL will be present in a vaccine in the range 1 µg - 100 µg, preferably 10 µg - 50 µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

As a further exemplary alternative, the fusion protein gE-IE63, can be encapsulated within microparticles such as liposomes. In yet another exemplary alternative, the VZV fusion gE-IE63 protein can be conjugated to an immunostimulating macromolecule, such as killed Bordetella or a tetanus toxoid.

Vaccine preparation is generally described in *New Trends and Developments in Vaccines*, Voller et al. (eds), University Park Press, Baltimore, Maryland, 1978. Encapsulation within liposomes is described by Fullerton, US Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, US Patent 4,372,954 and Armor et al., US Patent 4,474,757. Use of Quil A is disclosed by Dalsgaard et al., Acta Vet Scand, 18:349 (1977). 3D-MPL is available from Ribi immunochem, USA, and is disclosed in British Patent Application No. 2220211 and US Patent 4912094. QS21 is disclosed in US patent No. 5057540.

The use of the polynucleotide of the invention in genetic immunisation will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., *Hum Mol Genet* 1992, 1:363, Manthorpe et al., *Hum. Gene Ther.* 1993:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., *J Biol Chem* 1989: 264, 1985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS*, 1986:83, 9551), or with other facilitators encapsulation of DNA in various forms of liposomes (Kaneda et al., *Science* 1989:243,375), particle bombardment (Tang et al., *Nature* 1992, 356:152, Eisenbraun et al., *DNA Cell Biol* 1993, 12:791) and in vivo infection using cloned retroviral vectors (Seegar et al, *PNAS* 1984:81,5849) or non-infectious, recombinant viral vectors, such as Semliki Forest helper 2 system (Berglund) et al *Bio/Technology* 1993, 11:916-920. Suitable promoters for muscle transfection include CMV, RSV, Sra, actin, MCK, alpha globin, adenovirus and dihydrofolate reductase. A preferred way of delivery of a polynucleotide in accordance with the invention utilises gold particles delivered by means of a gene gun (US 5100792, US 5036006, and 4945050).

The polynucleotide compositions of the invention may be administered using the dosages and routes of administration described in WO 90/11092. It will be apparent however that the precise dosage will depend on factors such as the weight, sex, mode of administration,

general health of the patient and the condition to be treated. Nonetheless for intramuscular use the dosages to be employed will typically be in the range of 0.05µg/kg to about 50 mg/kg, more typically from about 0.1 to 10 mg/kg. Subcutaneous, epidermal, intradermal or mucosal administration may be advantageous, due to the ability to induce high levels of CTL.

The vaccine of the present invention may additionally contain other antigenic components such as VZV gE, gB, gH, gI or gC or their truncated or anchorless derivatives or other immediate early proteins such as IE62 protein. In particular truncated gE, gB or gH as disclosed in European Patent application published under No. 0 405 867.

In a preferred embodiment of the present invention there is provided a vaccine composition comprising an anchorless VZV glycoprotein, especially gE, in combination with the fusion gE-IE63 protein.

By anchorless VZV glycoprotein is meant, a VZV glycoprotein derivative which is devoid of substantially all of the C-terminal anchor region and which allows for secretion when expressed in mammalian cells. Such proteins are described in EP-A-0405867.

In an alternative embodiment, a polynucleotide capable of expressing the gE-IE63 fusion protein may be used directly as a nucleic acid vaccine. Preferably the polynucleotide is DNA. In such cases, the polynucleotide will be under the control of a suitable promoter.

RNA immunisation may also be used. RNA in such cases, may be prepared in accordance with the methods of WO92/10578.

Examples

I. MATERIAL AND METHODS

I.1. Cloning and expression

I.1.a. Cloning and expression of amino extended ie63

The expression plasmid pNIV2094, encoding ie63, was constructed in 5 steps:

- A 4027 bp *SspI-SspI* DNA fragment was excised and recovered from the pUC19 plasmid carrying the genomic *EcoRI*A fragment of VZV, blunted and then inserted into the pUC19 *HincII* site, yielding the intermediate construct pNIV2032.
- A 832 bp *StyI-StyI* DNA fragment was recovered from pNIV2032, blunted and inserted into the pUC19 *HincII* site, yielding the intermediate construct pNIV2052.
- A pair of synthetic oligonucleotides was inserted between *NcoI* and *BamHI* sites of pNIV2052 to restore the 3' end of the ie63 coding sequence including the stop codon and to create a 3' *BamHI* site. This intermediate construct has been named pNIV2037.
- A 856 bp *PstI-BamHI* DNA piece was recovered from pNIV2037, blunted and inserted in the *EcoRV* site of the *E.coli* expression vector pMG81kn.
- The last step involved an excision by *NdeI* restriction followed by ligation to yield the final plasmid pNIV2094.

The restriction map of pNIV2094 is shown in Figure 1A. By construction, the protein when expressed via plasmid pNIV2094 carries 10 excedentary N-terminal amino acids corresponding to the amino-terminal part of NS1 protein: MLTSTRWSKD.

For expression, plasmid pNIV2094 was used to transform *E.coli* strain AR58 [*Fr⁺m⁺rpsL31 gal2 recA13 galE : Tn10(Tet^R)Δ8(chlD-pgl)*]. Recombinant bacteria were then grown in rich LB medium at 30°C up to and OD₆₂₀ of 0.35, then induced at 42°C for two hours to achieve expression and production of the protein.

Total proteins from induced and non-induced bacteria were separated onto SDS-PAGE; the data show that the induced bacteria produce a 43 kDa protein at about 5% of total proteins. This 43 kDa species is recognized in Western blot by a mouse polyclonal antiserum raised against an ie63 peptide (residues 70-86) and by a rabbit polyclonal antiserum raised against the complete ie63 protein.

I.1.b. Cloning and expression of authentic ie63 protein

The expression plasmid pNIV4807, encoding the authentic ie63 protein, has been constructed as follows:

90 10

Plasmid pNIV2094 was restricted with *Afl*III and *Hpa*I to eliminate a DNA fragment comprising 18 nucleotides upstream to the ATG initiation codon and 66 nucleotides downstream to the ATG codon. It was replaced by a synthetic oligonucleotidic pair to reconstitute the missing part of the promoter and the 5' end of the ie63 coding sequence. The sequence of the oligonucleotides reads as follows :

5' TTAAGGAGGATATAACATATGTTTTGCACCTACCGGCTACGCGGGGCGACTCGTCCGA
 3' CCTCTATATTGTATACAAAACGTGGAGTGGCCGATGCGCCCCGCTGAGCAGGCT
 GTCAAAACCCGGGGCATCGGTTGATGTT 3'
 CAGTTTTGGGCCCCGTAGCCAACACTACAA 5'

For expression, plasmid pNIV4807 was used to transform *E.coli* strain AR58 as described above.

I.1.c. Cloning and expression of the DNA encoding the fusion protein gE-ie63, pNIV4801

- The starting material for this construction consisted 1) of pNIV2088 (a mammalian expression vector, pEE14, carrying in its *Hind*III/*Sma*I sites, a blunted *Hind*III/*Bcl*II DNA piece corresponding to the coding sequence of anchor-less gE protein), and 2) of pNIV2097, a plasmid carrying a gE-gB-ie63 fusion. This plasmid is irrelevant to the present application, its purpose is only to provide convenient access to ie63 DNA for simple restrictions.
- Plasmid pNIV4801 was constructed by ligating four DNA fragments:
 - a 10.258 bp *Eco*RI/*Bst*EII DNA piece excised from pNIV2088, covering the sequence of the pEE14 vector, an untranslated genomic sequence corresponding to the 56 bp preceding ORF68 in the VZV genome and the sequence coding for amino acids 1-316 of gE.
 - a 674 bp *Bst*EII/*Ava*II fragment also recovered from pNIV2088 and specifying amino acids 317 to 540 of gE.
 - a *Ava*II-*Nsp*I 15/22 bp synthetic oligonucleotidic pair, coding for amino acids 541 to 546 of gE and followed by 3 bp allowing, via the *Nsp*I site, the *in frame* fusion with the first ATG codon of ie63, which thus now encodes an internal methionine.
 5' GACCGGAGGGCTTGCAGACATG 3'
 3' GCCTCCCGAACGTCT 5'
 - a 841 bp *Nsp*I/*Eco*RI DNA fragment recovered from pNIV2097 and coding for amino acids 2 to 278 of ie63.

The final expression plasmid, pNIV4801, thus, codes for a gE (signal⁺, anchor⁻, aa 1-546) x ie63(aa1-aa278) fusion protein. (Fig. 1B).

Note: pNIV4801, in the SBBio nomenclature, is referred to as pRIT14583.

For expression, plasmid pNIV4801 was used to transfect CHOK1 cells. After a 25 μ M MSX selection, clones supernatants were tested in Western blot with a monoclonal antibody raised against gE.

I.2. Cultures

I.2.a. Fermentations

10 L fermentors were inoculated with precultures of recombinant *E.coli* bacteria carrying plasmids pNIV2094 or pNIV4807. The culture medium, Opti 3X-glucose, consists of 0.66% bactotryptone; 1.5% yeast extract; 1.5% NaCl; 0.3% KH_2PO_4 ; 1% glucose and 20mg/L kanamycin. Fermentation proceeded at 30°C and pH 7.0. When the culture reached an OD_{620} of 13, the temperature was shifted to 42°C and induction of ie63 expression was maintained for 2 hours. Bacteria were then harvested by centrifugation for 30 min at 12000 rpm and stored at -20°C.

I.2.b. Cultures in cell factories of a CHOK1 recombinant cell line (gE-ie63 n°22) expressing the gE-ie63 fusion protein.

The recombinant CHOK1 cell line, gE-ie63 n°22, was cultivated in 6000 cm² cell factories (NUNC) in GMEM medium (Gibco) supplemented with 2% fetal calf serum (Gibco) and 2mM sodium butyrate. Medium harvesting (1.700ml) was performed every three days. Pools of four successive harvests, stored at -20°C before use, were engaged in the purification process (see below).

I.3. Purification

I.3.a. Purification of amino-extended ie63 protein

Bacteria (60g), harvested from fermentors, were resuspended in buffer 20mM Tris-HCl pH7.5, 1mM aprotinin and 1mM AEBSF, then lysed in a French pressure cell (2 passages at 15000 psi). Cell debris were eliminated by a first centrifugation (30 min at 15000 rpm, SS34 rotor) and the resulting supernatant was further clarified by ultracentrifugation (1 hour at 45000 rpm, rotor 60TI). The clear lysate was then applied onto a Q-sepharose fast flow column (15 x 2.6 cm Pharmacia) equilibrated in 20mM Tris-HCl pH 7.5 buffer. After extensive washing of the column with the same buffer, bound proteins were eluted stepwise from the column by increasing NaCl concentration in the buffer.

The ie63 protein elutes from the column at 400mM NaCl; the ie63-containing fractions were immediately applied onto a Blue Trisacryl Plus LS column (7 x 2,6 cm, Biosepra), conditioned in 20mM Tris-HCl pH 7.5 buffer. After loading and washing the column extensively with the same buffer, the resin was eluted stepwise with increasing NaCl concentrations (500, 1000 and 2000 mM). The ie63 protein was recovered in fractions 1000 and 2000 mM NaCl. These were pooled and applied onto a Ni^{2+} chelate sepharose fast flow column (12 x 1 cm, Pharmacia), conditioned in PBS buffer, pH 7.5 containing 500mM NaCl. After washing of the column with starting buffer, proteins were eluted by increasing imidazole concentration in the column buffer. The ie63 protein was recovered from 20 and 30mM imidazole fractions and its purity estimated at more than 90%.

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I.3.b. Purification of gE-ie63 fusion protein

Spent culture medium (7l, four harvests) was applied onto a Q-sepharose fast flow column (15 x 2,6 cm Pharmacia) conditioned in 20 mM Tris-HCl pH 7.5. After washing, proteins were released from the column by stepwise addition of NaCl in conditioning buffer. The gE-ie63 protein was recovered in the 1000 mM NaCl fraction. This material was then adjusted to 1000 mM $(\text{NH}_4)_2\text{SO}_4$ and applied onto a butyl TSK column (8 x 2,6 cm Tosohaas), conditioned in PBS buffer pH 7.5, 1000 mM $(\text{NH}_4)_2\text{SO}_4$. The column was first washed with PBS buffer supplemented with 200 mM $(\text{NH}_4)_2\text{SO}_4$ and then eluted with water to recover the gE-ie63 protein. The enriched fraction was further purified onto a Ni^{2+} chelate sepharose fast flow column (12x1 cm, Pharmacia) conditioned in PBS buffer, pH 7.5, 500mM NaCl. After washing of the column, bound proteins were eluted with increasing imidazole concentrations. The gE-ie63 protein eluted at 100 mM imidazole and was pure at more than 80%.

I.3.c. Purification of gE proteins

The purification protocol of the anchor-less gE protein, produced by engineered CHOK1 cells, has been published by us in *Virus Research* 40, 199-204 (1996).

I.4. Immunology

I.4.a. Animals and immunisations

8 weeks old female BalbC mice and 500 to 600 g female Dunkin Hartley guinea pigs were obtained from Iffa Credo.

First immunisation experiment

Four groups of 4 mice were twice immunised via footpads at 28 days interval. Bleedings were performed on days 28 and 42. On day 42, mice were sacrificed to recover spleens and popliteal lymph nodes. Groups 1 and 2 received 4 μg of amino-extended ie63 adjuvanted either with SBAS2 or SBAS1C. Groups 3 and 4 received adjuvants only.

Second immunisation experiment

Five groups of 6 guinea pigs were twice immunised subcutaneously at 28 days interval. Bleedings occurred on days 28, 42 and 72. All antigens (8 μg /guinea pig) were adjuvanted with adjuvant X (SB). Group 1 received protein gE, group 2 received amino-extended ie63 protein, group 3, the fusion protein gE-ie63, group 4 gE and amino-extended ie63 separately, and group 5 the adjuvant.

Third immunisation experiment

Five groups of 6 mice were twice immunised via footpads at 28 days interval. Bleedings occurred at days 28 and 42. On day 42, mice were sacrificed to recover spleen and popliteal lymph nodes. All antigens (5 μg /mouse) were adjuvanted with adjuvant X (SB).

The five groups are identical to those described in the second immunisation experiment.

I.4.b. ELISA tests

a) IgG specific

Plates were coated with the gE protein (500ng/well), the gE-ie63 fusion protein (200ng/well) or the amino-extended ie63 protein (20ng/well), for 16 hrs at 4°C. Plates were then washed 5 times with 100µl per well of TBS-Tween (50mM Tris-HCl pH 7.5, 150mM NaCl, 0.1% Tween 80) and saturated for 1 hr at 37°C with 150µl of the same buffer supplemented with 1% BSA. 100 µl serial dilutions of sera to be tested were then incubated for 1 hr at 37°C. Plates were washed 5 times with TBS-Tween buffer and antigen-bound antibodies were detected with the second antibody (goat antimouse or goat antiguinea pig, Promega, USA) coupled to alkaline phosphatase (dilution 1/7500 in TBS-Tween buffer). The enzymatic activity was measured using the paranitrophenylphosphate substrate (Sigma) dissolved in diethanolamine buffer (pH 9.8). OD_{415nm} was measured in a Biorad Novapath ELISA reader.

b) IgG1 and IgG2a specific

Plates were coated and saturated as described above. 100µl serial dilutions of sera to be tested were incubated for 1 hr at 37°C. Antigen-specific IgG1 and IgG2a were detected with biotin-labelled antibodies against IgG1 and IgG2a (rat antimouse, dilution 1/7000 in TBS-Tween buffer and 1% BSA, Biosource). Phosphatase alkaline-conjugated streptavidin (1/1000 dilution, Amersham) was then added to each well. Measure of the enzymatic activity proceeded as described above.

I.4.c. Neutralisation assays

VZV virus (Webster strain) was diluted up to a concentration of $4 \cdot 10^2$ pfu/ml in VZV buffer (PBS pH 7.1; 5% saccharose, 1% glutamate, 10% Fetal Calf serum). 100µl of the suspension were incubated for 1 hr at 37°C with 100µl of serial dilutions of sera to be tested (after complement inactivation) in VZV buffer. 2µl of guinea pig serum (Gibco-Grand Island) were added to each well. The mixture was then added onto confluent MRC5 cells and incubation proceeded for 2 hrs at room temperature in the dark. 800 µl of culture medium (BME, 2% FCS) were added and cells were allowed to grow for 7 days. At this time, spent medium was discarded and cells were fixed then stained with Coomassie blue for 10 minutes (0.5% R250; 45% methanol, 10% acetic acid). The neutralising titre was expressed as the reciprocal of the serum dilution leading to a 50% reduction in the number of viral plaques.

I.4.d. Coupling gE and amino-extended ie63 to sepharose, depletion assays

gE and ie63 proteins were coupled to activated CH4B sepharose (Pharmacia, Sweden). One milligram of gE or amino-extended ie63, preably dialysed against the coupling buffer (0.1M NaHCO₃, 0.5M NaCl, pH 8) was incubated with 3ml of activated resin and 9ml of coupling buffer. The coupling efficiency was measured by dosing residual gE or ie63 proteins by ELISA. After coupling, residual reactive groups were blocked with 0.1M Tris-HCl, pH 8 and the gel was washed three times at pH 4 and pH 8, alternatively. For depletion assays, 100µl of the serum to be tested were diluted five fold in PBS buffer and incubated with 100µl of coupled gel for 16 hrs at 4°C. The supernatant was collected and the gel washed twice with

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250 µl of PBS. The negative control for depletion was achieved on BSA-coupled sepharose. After depletion, residual antibodies were undetectable in ELISA.

I.4.e. Proliferation assays

Lymphocytes were isolated from spleens and popliteal lymph nodes. $4 \cdot 10^5$ cells, in triplicate, were incubated with serial dilutions of antigens in 96 well plates (Nunc) for 4 days (10 base 2 dilutions of the antigen were tested, starting from a concentration of 25 µg/ml). Cell proliferation was shown by measuring ^3H -thymidine incorporation during 16 hours of proliferation. Negative controls were irrelevant antigens, obtained from bacterial or CHO sources and purified similarly as the antigens of interest.

II. RESULTS AND DISCUSSION

II.1. Cloning and expression of ie63

The expression level for authentic ie63 was of the same order of magnitude than the one measured for amino-extended ie63. For the immunological studies, the amino-extended ie63 protein was used since authentic ie63 was not available at that time.

II.2. Purifications of the proteins

II.2.a. Fermentation and purification of amino-extended ie63 protein

A fermentation protocol, suitable for the production of large quantities of recombinant ie63, has been developed starting from bacteria transformed with pNIV2094. Induction conditions for expression have been optimised for 10L batch fermentations. Western blot analysis shows that the addition of 10g/L of glucose during the induction period extends bacterial growth and triples the expression level for ie63. Addition of 2g/L yeast extract had no such effect. pH regulation during induction appears crucial for maintaining ie63 in solution, preventing aggregation. At pH 7.0, 70% of ie63 protein remain soluble, whereas this percentage drops to 20% in absence of pH regulation. The ie63 protein was purified in three steps; an anion exchange, a Blue trisacryl step and a metal chelate step. The final yield of the purification process was 250 µg ie63 per gram of packed bacteria. The molecular weight, estimated by SDS-PAGE, was 40 kDa. Lower Mw bands, also recognised by Western blot with anti ie63 polyclonal antibodies, represent degraded forms of the protein.

II.2.b. Purification of the fusion protein gE-ie63

The fusion protein gE-ie63 was purified in three steps: an anion exchange, an hydrophobic interaction and a metal chelate step. The protocol closely resembles the one used for gE, except that the fusion protein elutes from the Q-sepharose column at a higher NaCl concentration (1000 mM versus 300 mM). The final yield was 1 mg per liter of cell factory-derived spent medium. The fusion protein migrated on SDS-PAGE as a wide band of 180 kDa, together with finer 130, 85 and 75 kDa subspecies. All these products were recognized,

in Western blot, by the monoclonal antibody β D6 raised against gE and by rabbit polyclonal antibodies raised against ie63. The amino terminal sequence of these 4 bands was determined by Edman degradation; all sequences proved identical and corresponded to the N-terminus of gE protein. Apparently, lower Mw products resulted from the degradation of the ie63 moiety of the fusion protein.

II.3. Immunology

II.3.a. First Experiment

Groups of mice and immunisation schedule have been described in the Material and Methods section. It was found that the mean titer of specific IgGs was significantly lower for group 1 (ie63 + SBAS2) than for group 2 (ie63 + SBAS1C), i.e. 8864 versus 21148 (see figure 2). Control groups gave ELISA signals below the detection threshold. Mean titer for IgG1 and IgG2a in sera of immunised animals were determined (see figure 3 and table 1 below).

Table 1

	Group 1	Group 2
IgG1	11924	18437
IgG2a	7509	17643

Again, the mean titer for IgG1 and IgG2a in group 1 was lower than for group 2. However, the IgG1/IgG2a ratio is higher in group 1 than in group 2 (1.58 versus 1.05), which seems to indicate that the formulation of ie63 with SBAS2 biased the immune response towards a TH2 type, more clearly than with the SBAS1C adjuvant.

The lymphoproliferative response was measured after 3, 4 or 5 days incubation of the lymphocytes with the antigen. Responses were shown in Figure 4 (spleen cells derived from all 4 mice in each group were pooled as well as cells derived from popliteal lymph nodes). Proliferation was observed only for group 1 (ie63 + SBAS2).

At high concentrations of antigen, the proliferation index was around 10 for both spleens and popliteal lymph nodes. Although 3 days are sufficient to read the response for spleen cells, experiments were generally performed after 5 days, the necessary time span for the read out of popliteal lymph nodes. The failure to observe a lymphoproliferative response in group 2 appears to be due to a technical problem since the humoral response in this group was quite satisfactory.

In order to check the secretion of IFN γ and IL-5, two cytokines typical respectively of TH1 and TH2 responses, an aliquot of culture supernatants was tested in SBBio. As seen in Figure 5, both IFN γ and IL-5 were found, in agreement with the presence of IgG1 and IgG2a in mice sera. The highest concentrations for the two cytokines were obtained after 5 days of stimulation with ie63.

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II.3.b. Second experiment

The humoral response, induced by the injection of proteins gE, ie63 or gE-ie63 was studied in guinea pigs. Groups and immunisation schedule have been detailed in the Materials and Methods section. ELISA titers and curves are shown in Table 2 and Figure 6.

As a rule, mean ELISA titers were lower at day 72 (44 days post 2nd injection) than at day 42 (14 days post 2nd injection). The humoral response appeared higher with the gE + ie63 formulation than with the gE formulation or the gE-ie63 fusion formulation when measured in gE ELISA.

The same observation was done when comparing the gE + ie63 formulation to the others as measured in ie63 ELISA. However, in the fusion ELISA, the gE + ie63 formulation gave titers of the same order of magnitude than the gE-ie63 fusion formulation.

Neutralisation assays were performed with guinea pigs sera, for the three bleedings (see Material and Methods). The mean neutralising titers are shown in Figure 6.

The gE-ie63 fusion protein induced a titer in neutralising antibodies higher than those measured for gE or for gE + ie63, injected simultaneously. To the contrary of ELISA titers, neutralising titers did not decrease between days 42 and 72. Note that injection of ie63 did not induce detectable neutralising antibodies. This is also apparent when comparing neutralising titers induced by gE and gE + ie63, which for each bleeding are similar.

To check the significance of the difference between neutralising titers induced by gE and gE-ie63 fusion, sera from the 2nd and 3rd bleeding of guinea pigs immunised with the fusion protein were depleted on sepharose-immobilised gE or ie63. ELISA curves for sera from group 3, before and after depletion, are shown in Fig. 7 and 8. Depleted sera were then assayed for neutralisation, as shown in Table 3. Depletion on immobilised ie63 did not lead to any significant decrease of the neutralising titer whereas depletion on the gE sepharose column abolished the neutralising capacity. It can thus be concluded that the fusion between gE and ie63 did not create new epitopes.

II.3.c. Third experiment

Groups and immunisation schedule were identical than in the second experiment. The injected dose was however different (5µg) and the animals were mice instead of guinea pigs. Mean ELISA titers for specific IgGs were again measured. The gE + ie63 formulation did not induce a higher response (Figure 9) as was seen with guinea pigs. Whatever the ELISA assay, the titer for this formulation was the lowest of all.

IgG1 and IgG2a titers were similar in all three cases of formulation (Figure 9). Spleen cells and popliteal lymph nodes were stimulated for 5 days with decreasing concentrations of gE, ie63 or gE-ie63 fusion.

For group 1 (gE), no significant proliferation occurred by *in vitro* stimulation with gE.

For group 2 (ie63), lymphocytes proliferated in function of the dose of ie63 (Figure 10). Using an irrelevant protein as negative stimulator, no proliferation was detected, indicating

that the stimulation by ie63 was specific, confirming data obtained in the first experiment (II.3.a). The fusion protein induced a significant proliferation in only 1 out of 6 mice.

For group 3 (gE-ie63 fusion), no stimulation occurred with gE. However, ie63 induced a significant proliferation of spleen and popliteal lymph nodes cells (Figure 11). This effect is specific since the injected fusion protein derived from CHO cells, whereas ie63 derives from bacteria.

Restimulation of lymphocytes with the fusion protein did not lead to interpretable results since the proliferative index was similar to that measured with an irrelevant protein also derived from CHO cells.

For group 4 (gE + ie63), results confirmed those obtained for groups 1, 2 and 3. gE did not induce proliferation, ie63 induced proliferation in 4 out of 6 mice. The data for gE-ie63 were not interpretable.

III. GENERAL CONCLUSION

Immunisation of mice and guinea pigs with the gE-ie63 fusion protein stimulates both humoral and cell-mediated responses (against ie63 and gE).

The level of these responses is similar to that observed by immunisation with individual gE and ie63 proteins. Since gE-ie63 can be purified more easily than gE and ie63, it has an obvious industrial advantage.

		Day 0	Day 30	2 nd experiment											
Guinea pig				Bleeding 1 (D = 30)				Bleeding 2 (D = 42)				Bleeding 3 (D = 72)			
				Elisa			Neutra	Elisa			Neutra	Elisa			Neutra
				gE	ie63	gE-ie63		gE	ie63	gE-ie63		gE	ie63	gE-ie63	
gE	c1			292			0	23684		9023	960	8337		2985	640
	c2			226			0	7973		1509	60	2549		875	240
	c3			359			60	13978		4727	60	4598		2474	480
	c4			118			30	15233		5909	1280	10829		2620	1920
	c5			263			0	9320		2096	120	1840		965	240
	c6			61			0	6351		1778	240	14300		706	480
ie63	c1				<20		0		4197	1026	0		1515	519	0
	c2				<20		0		263	83	0		<50	<50	0
	c3				<20		0		188	<50	0		51	<50	0
	c4				<20		0		696	116	30		112	<50	0
	c5				<20		0		70	80	0		<50	<50	0
	c6				<20		0		829	50	0		1207	278	0
gE-ie63	c1			69	81	105	0	5939	2433	6915	320	6131	788	2725	480
	c2			51	32	51	0	9120	4413	7380	480	2973	3266	3041	480
	c3			67	36	111	0	5142	2344	6455	0	2690	979	2194	240
	c4			313	86	250	30	15959	3479	12140	1920	10716	2014	5888	1920
	c5			1307	257	1152	0	20795	2622	16749	1920	13161	1495	6147	3840
	c6			456	116	346	0	5213	2418	5518	1920	7240	2293	3349	1920
gE+ie63	c1			1294	35		0	25805	7821	9669	60	15733	2544	5580	960
	c2			797	<20		0	22039	5670	13096	0	9913	934	4566	0
	c3			127	<20		0	2271	2270	1966	480	8534	972	4738	960
	c4			373	<20		240	36576	3009	13213	1920	1612	567	2915	1920
	c5			242	<20		0	18474	780	6618	30	12800	1369	+	+
	c6			2265	52		60	18854	5360	11145	240	3295	1101	1534	640
adjuvant	c1			<20	<20	<20	0	<50	<50	<50	0	<50		<50	0
	c2						0				0				0
	c3						0				0				0
	c4						0				0				0
	c5						0				0				0
	c6						0				0				0

Table 2

Neutralizing titer before and after depletion

Group 3	2 nd bleeding			3 rd bleeding		
	before	Depletion gE	Depletion ie63	before	Depletion gE	Depletion ie63
C1	320	30	320	480	20	480
C2	480	40	480	480	40	320
C3	0	0	0	240	0	240
C4	1920	40	1920	1920	60	1920
C5	1920	60	1920	3840	80	2560
C6	1920	40	1920	1920	40	1920

Table 3

Claims

1. A fusion protein comprising
 - (1) a Varicella Zoster Virus gE protein or an immunologically active fragment thereof fused to
 - (2) a different protein of Varicella Zoster Virus or an immunologically active fragment thereof.
2. A fusion protein as claimed in claim 1 comprising
 - (1) a Varicella Zoster Virus gE protein or an immunologically active fragment thereof fused to
 - (2) a Varicella Zoster Virus IE63 protein or an immunologically active fragment thereof.
3. Recombinant DNA or RNA encoding a fusion protein as claimed in any one of the preceding claims.
4. An expression vector or a recombinant live microorganism comprising recombinant DNA or RNA according to claim 3.
5. A host transformed with a nucleic acid according to claim 3 or with a vector according to claim 4.
6. A vaccine composition comprising a fusion protein as claimed in claim 1 or claim 2 and a pharmaceutically acceptable excipient.
7. A vaccine composition comprising a nucleic acid encoding the fusion protein of claim 1 or claim 2 and a pharmaceutically acceptable excipient.
8. A fusion protein as claimed in claim 1 or claim 2 for use in medicine.

9. A process for the production of a fusion protein according to claim 1 or claim 2 which process comprises expressing recombinant DNA encoding said protein in a host cell and recovering the protein.
10. Use of a fusion protein as claimed in claim 1 or claim 2 for the manufacture of a medicament for the prevention or amelioration of Varicella or Zoster.
11. A vaccine composition as claimed in claim 6 or 7 additionally comprising other VZV antigens.
12. A vaccine composition as claimed in claim 11 wherein the further Varicella Zoster Virus protein is selected from the group, gE, gB, gH, gI, gC or IE62 or an immunologically functional derivative thereof.
13. A vaccine composition as claimed in claim 6, 7, 11 or 12 additionally comprising an adjuvant.
14. A vaccine composition as claimed in claim 13 wherein the adjuvant preferentially induces a TH1 response.
15. A vaccine composition as claimed in claim 13 wherein the adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, a CpG oligonucleotide, aluminium hydroxide, aluminium phosphate and tocopherol, and a water in oil emulsion or a combination of two or more of the said adjuvants.
16. A method of treating a patient suffering from or susceptible to Varicella Zoster Virus infection or recurrence, comprising administering to a patient a safe and effective amount of a vaccine composition as claimed in any one of claims 6,7,11, 12, 13, 14 or 15.
17. A method of producing a vaccine composition as claimed in any one of claims 6,7, 11 or 12, comprising mixing said fusion protein or nucleic acid with a pharmaceutically acceptable excipient.

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18. A method as claimed in claim 17, wherein the excipient comprises an adjuvant as claimed in claim 13 - 15.

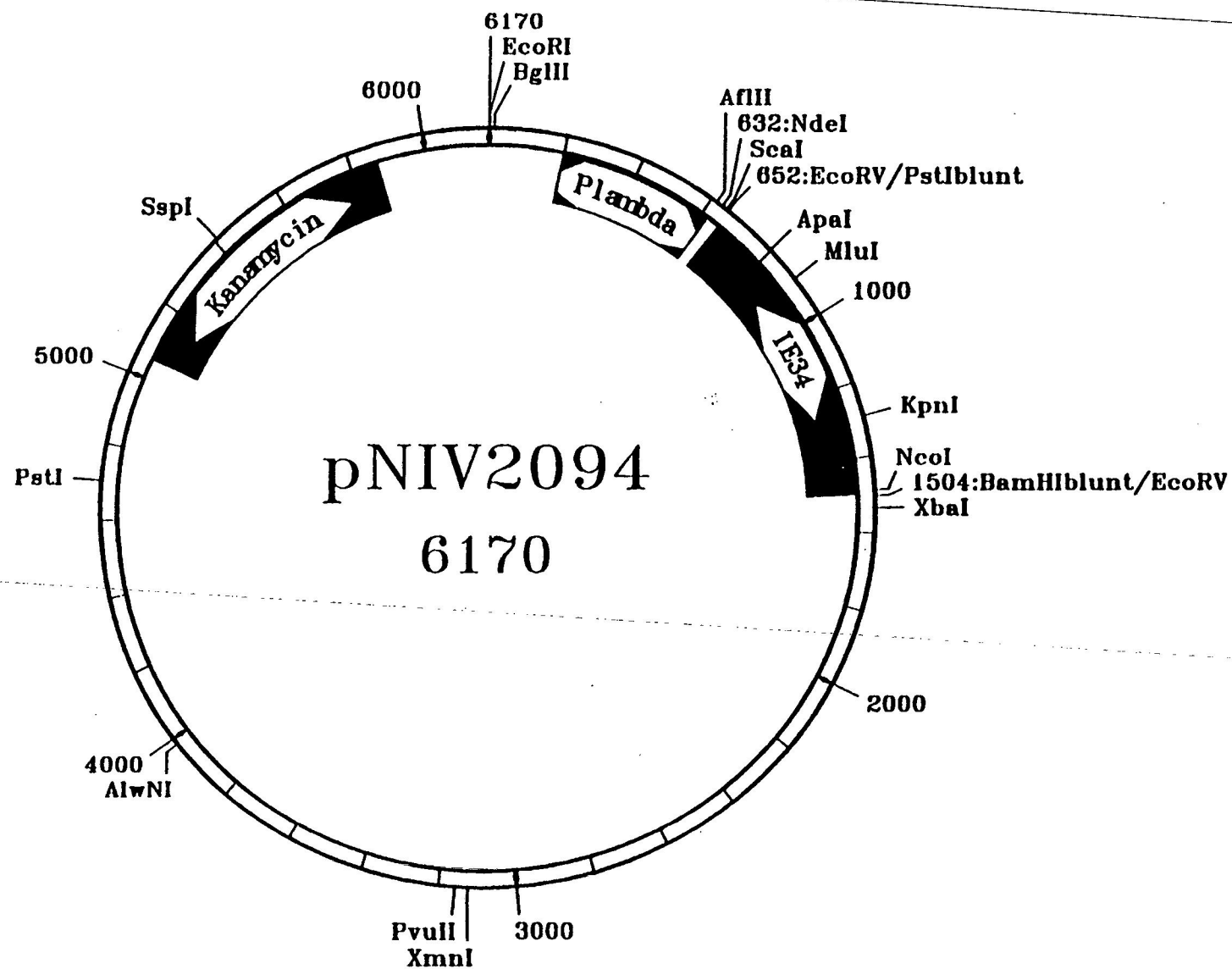
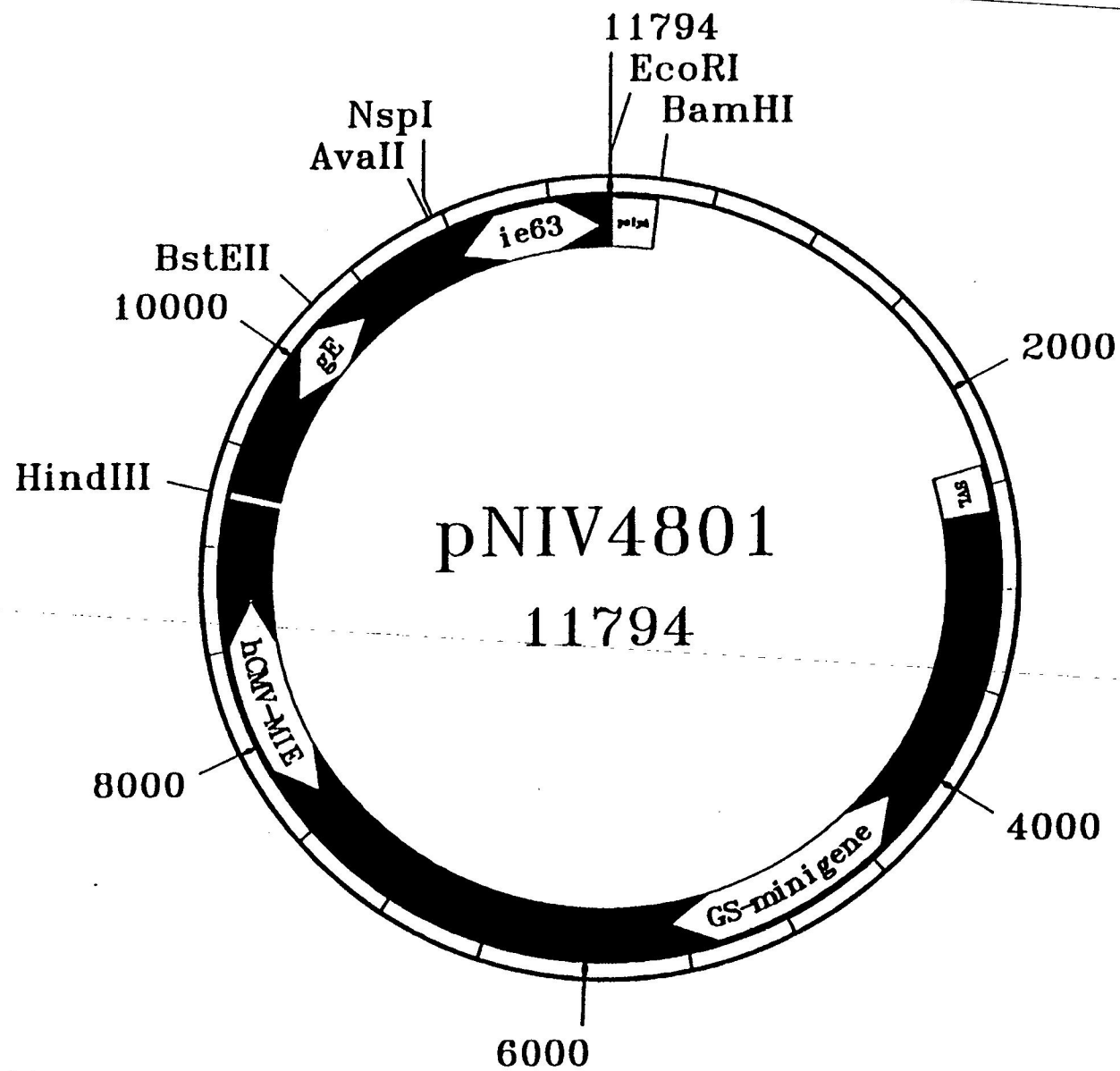


Figure 1A



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ie63 specific IgG
1st experiment

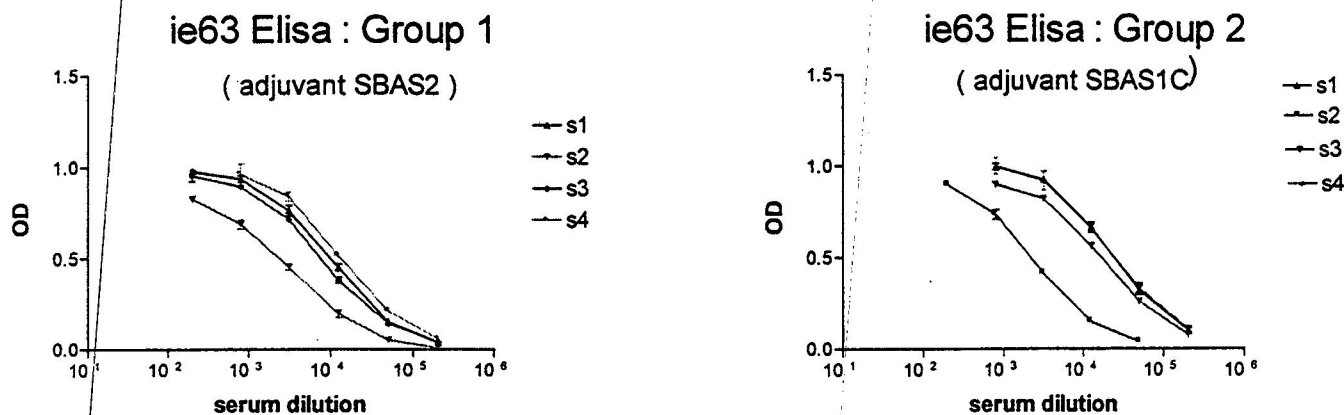


Figure 2

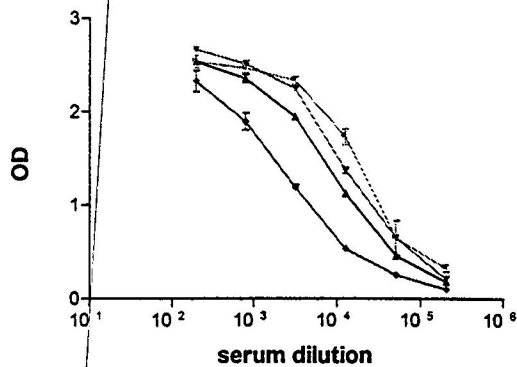
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100 ~~AD~~
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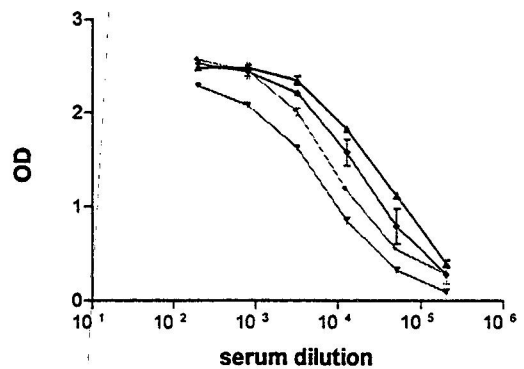
ie63 specific IgG1 and IgG2a

1st experiment

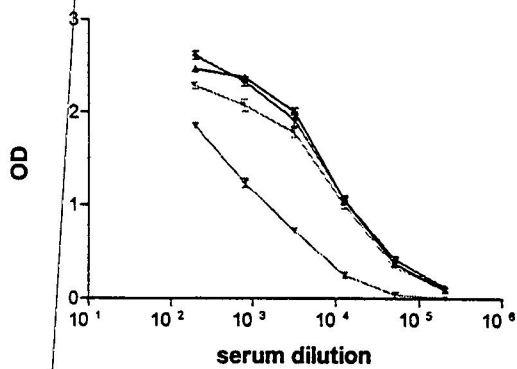
Elisa ie63 / IgG1 : Group 1



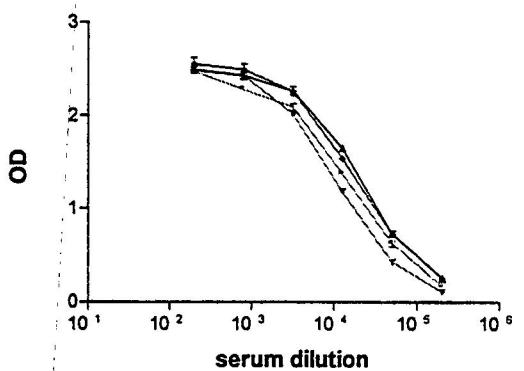
Elisa ie63 / IgG1 : Group 2



Elisa ie63 / IgG2a : Group 1



Elisa ie63 / IgG2a : Group 2



Elisa titer mean

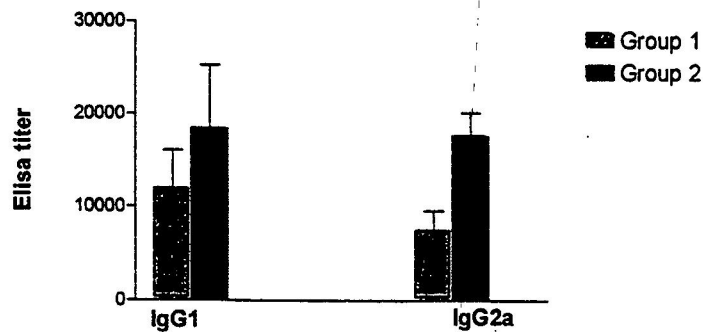


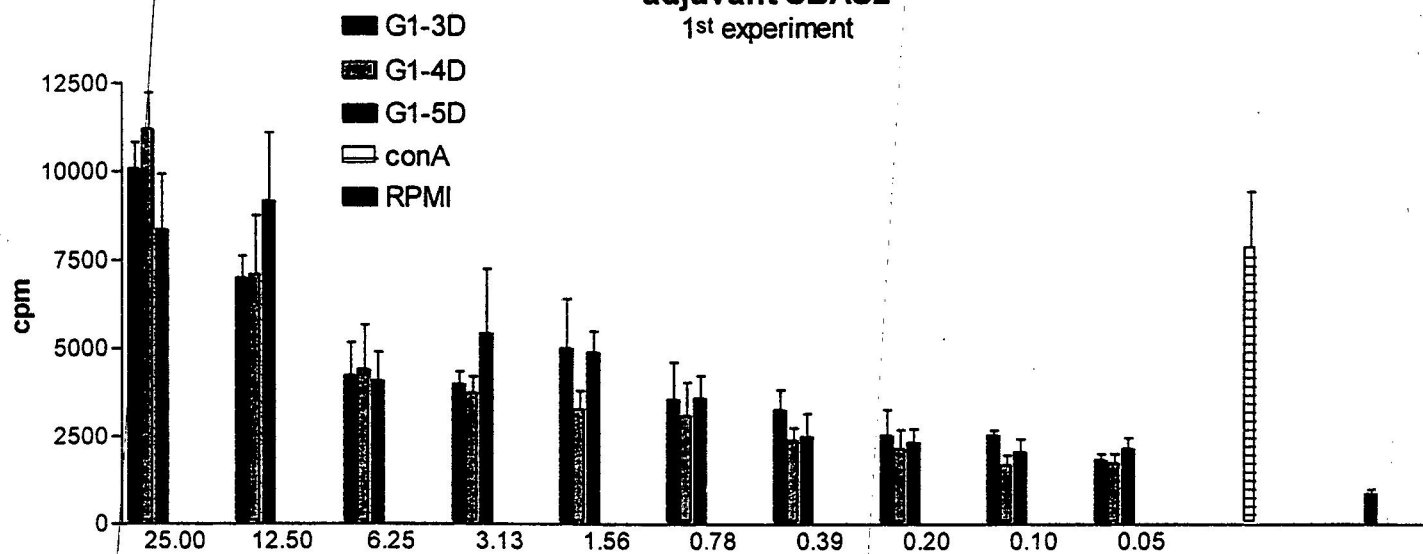
Figure 3

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Proliferation ie63 (spleen)
adjuvant SBAS2
1st experiment



Proliferation ie63 (Lymph nodes)
adjuvant SBAS2
1st experiment

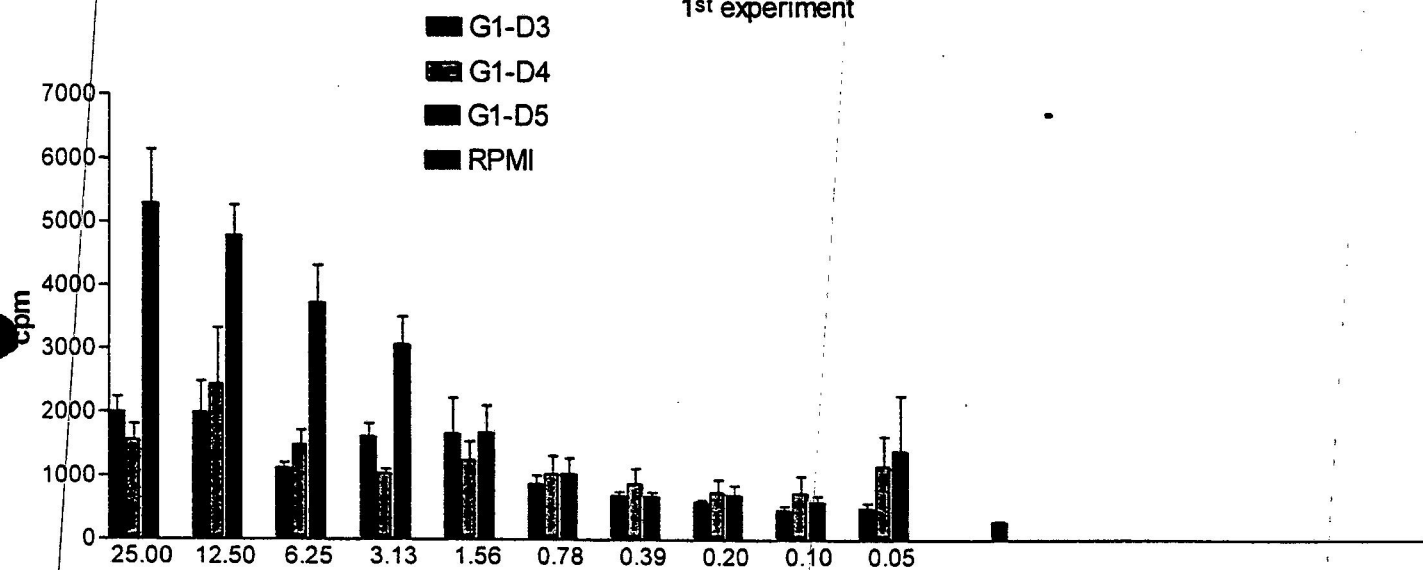


Figure 4

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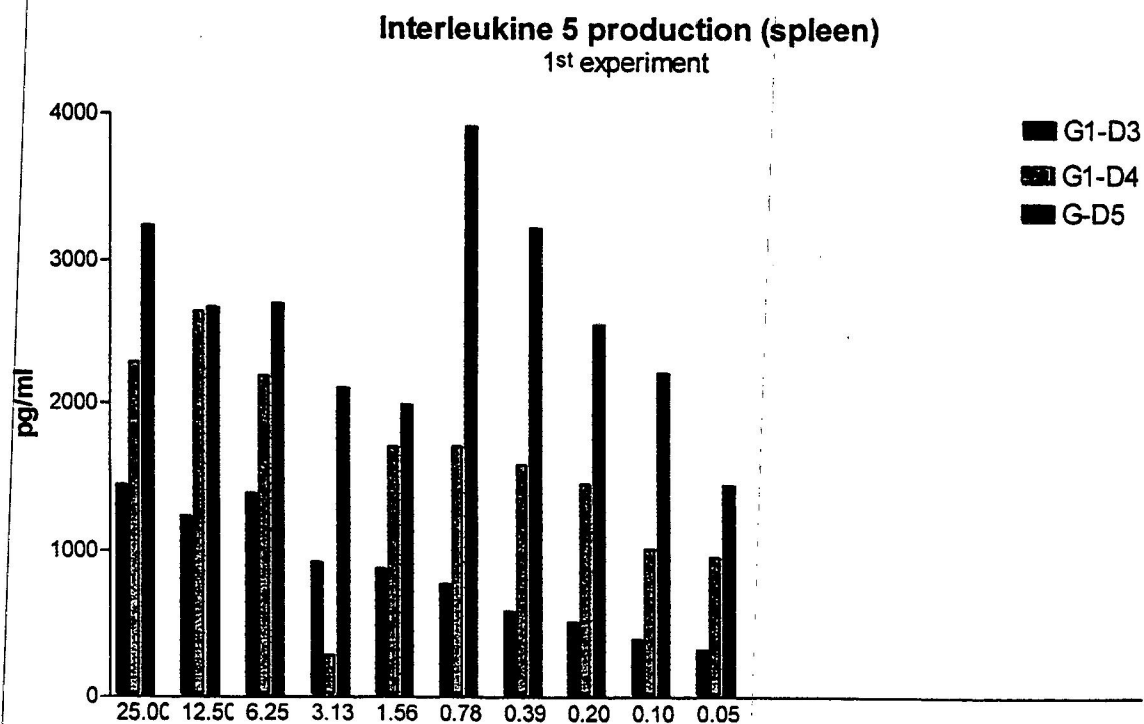
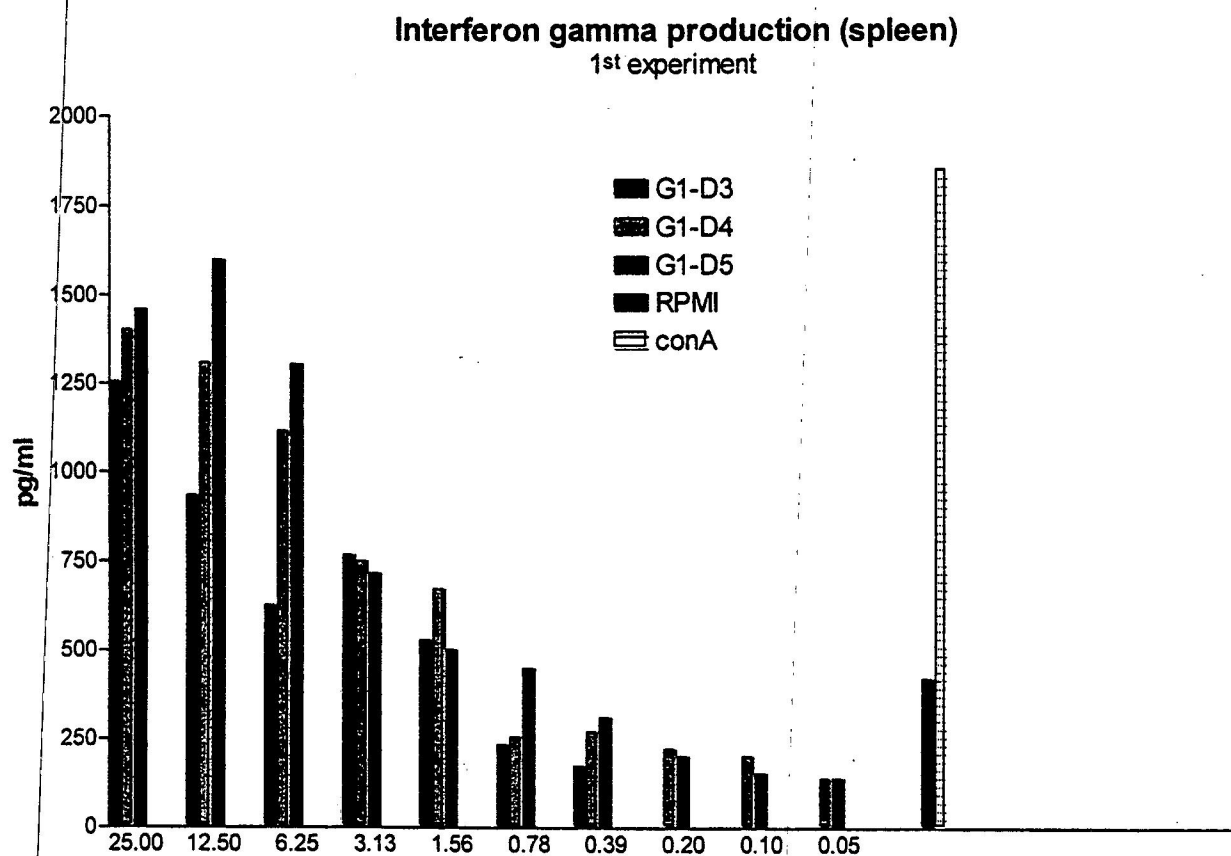


Figure 5

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gE, ie63 et gE-ie63 specific IgG

2nd experiment

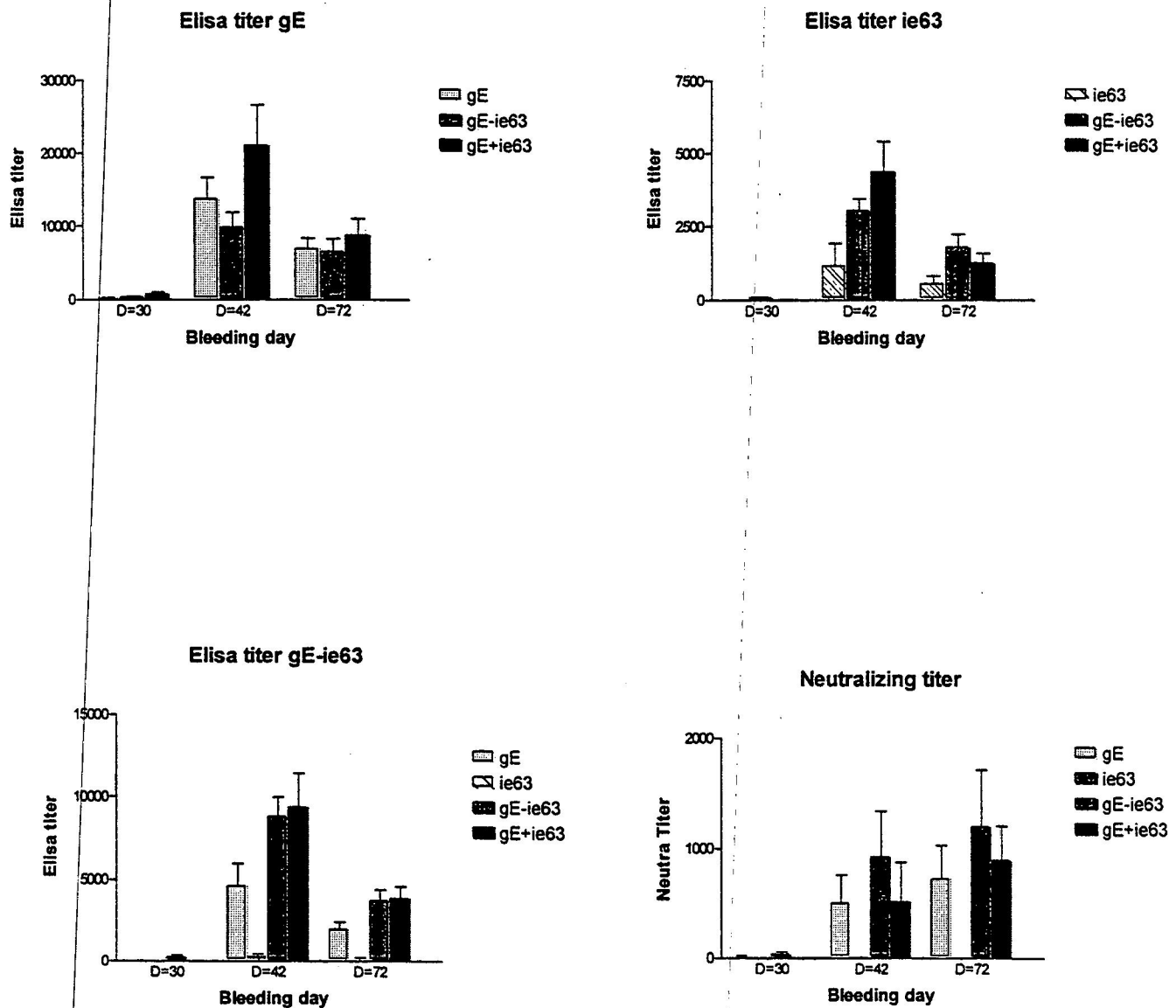


Figure 6

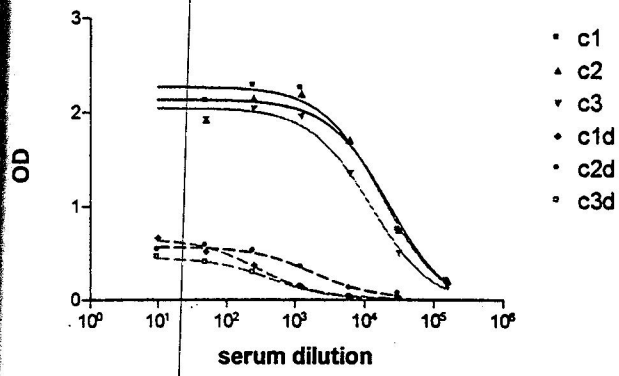
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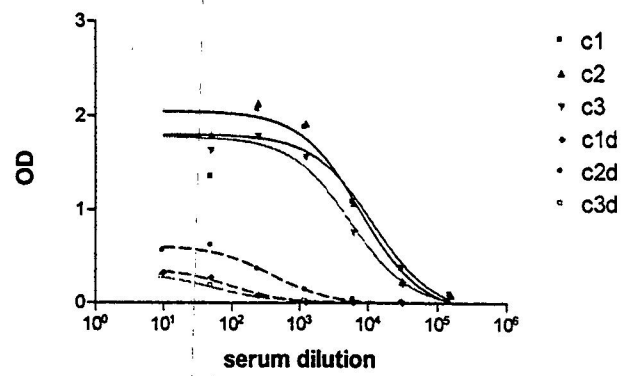
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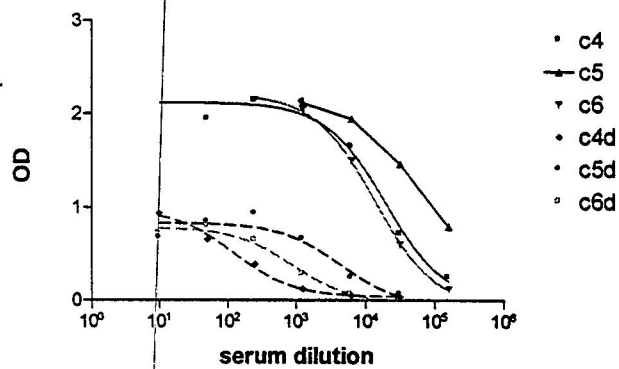
Elisa gE : Group 3
Bleeding 2



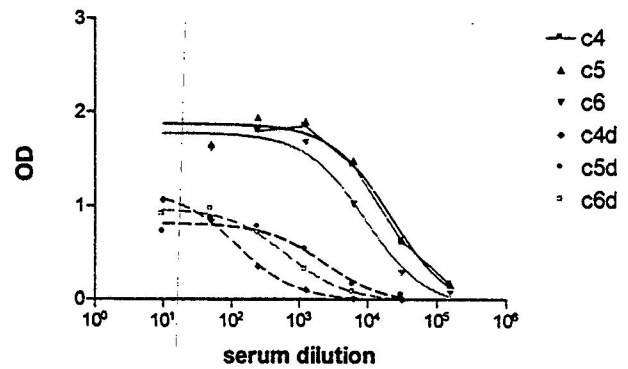
Elisa gE : Group 3
Bleeding 3



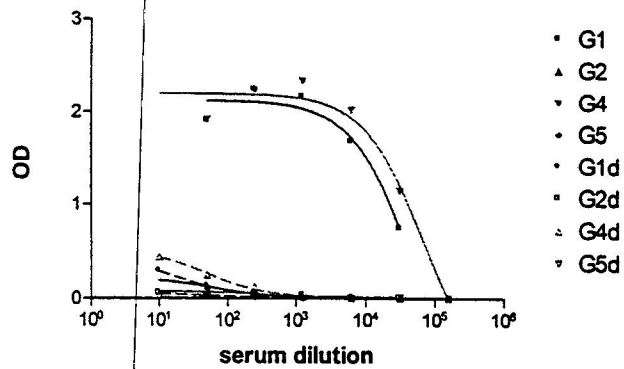
Elisa gE : Group 3
Bleeding 2



Elisa gE : Group 3
Bleeding 3



Elisa gE : Control group
Bleeding 2



Elisa gE : Control group
Bleeding 3

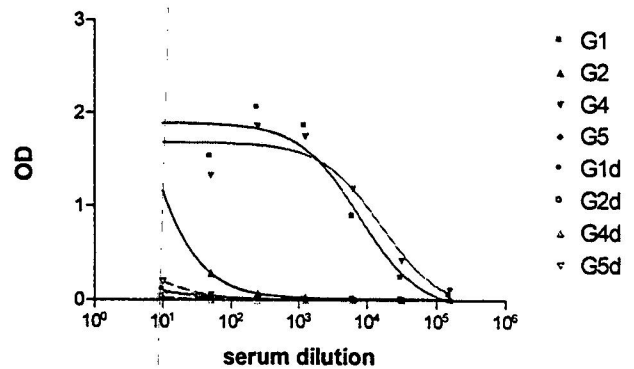


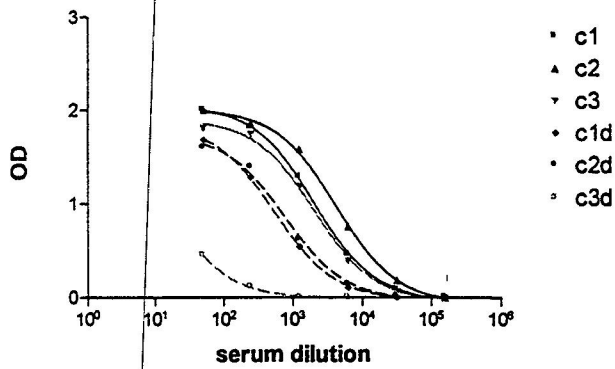
Figure 7

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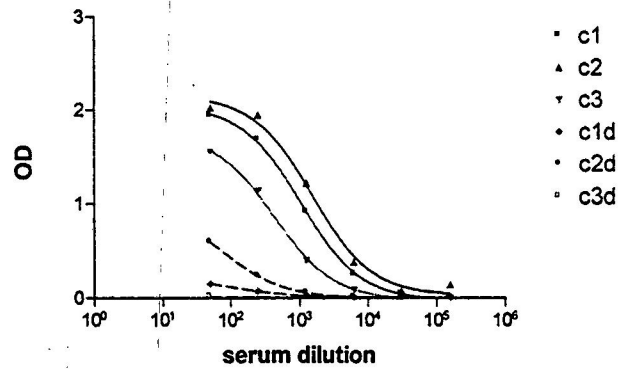
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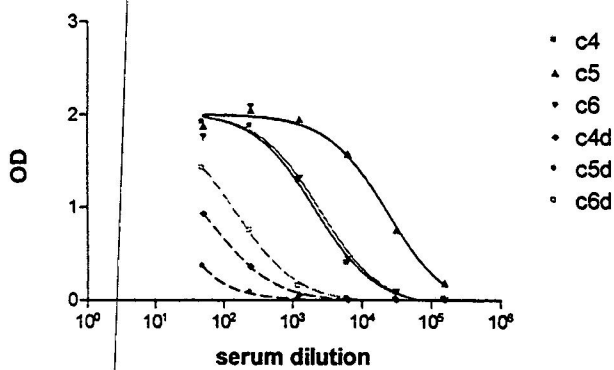
Elisa ie63 : Group 3
Bleeding 2



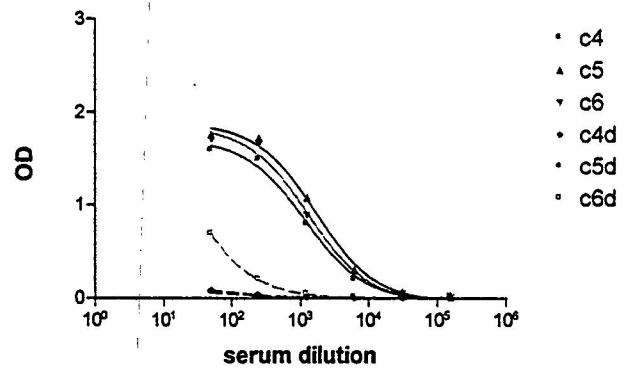
Elisa ie63 : Group 3
Bleeding 3



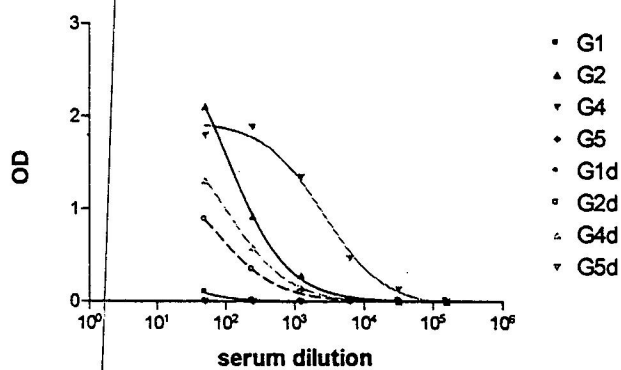
Elisa ie63 : Group 3
Bleeding 2



Elisa ie63 : Group 3
Bleeding 3



Elisa ie63 : Control group
Bleeding 2



Elisa ie63 : Control group
Bleeding3

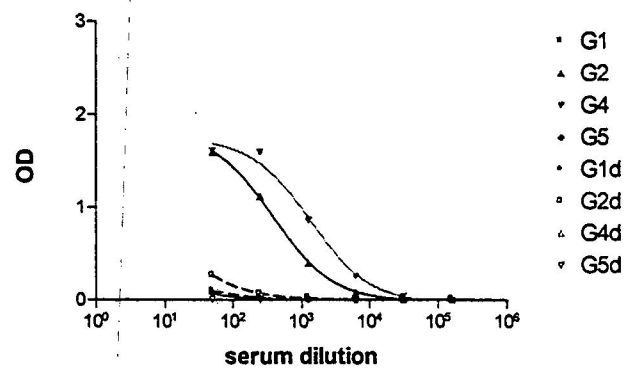


Figure 8

10/12

106
28
108

gE, ie63 et gE-ie63 specific IgG1 and IgG2a

3rd experiment

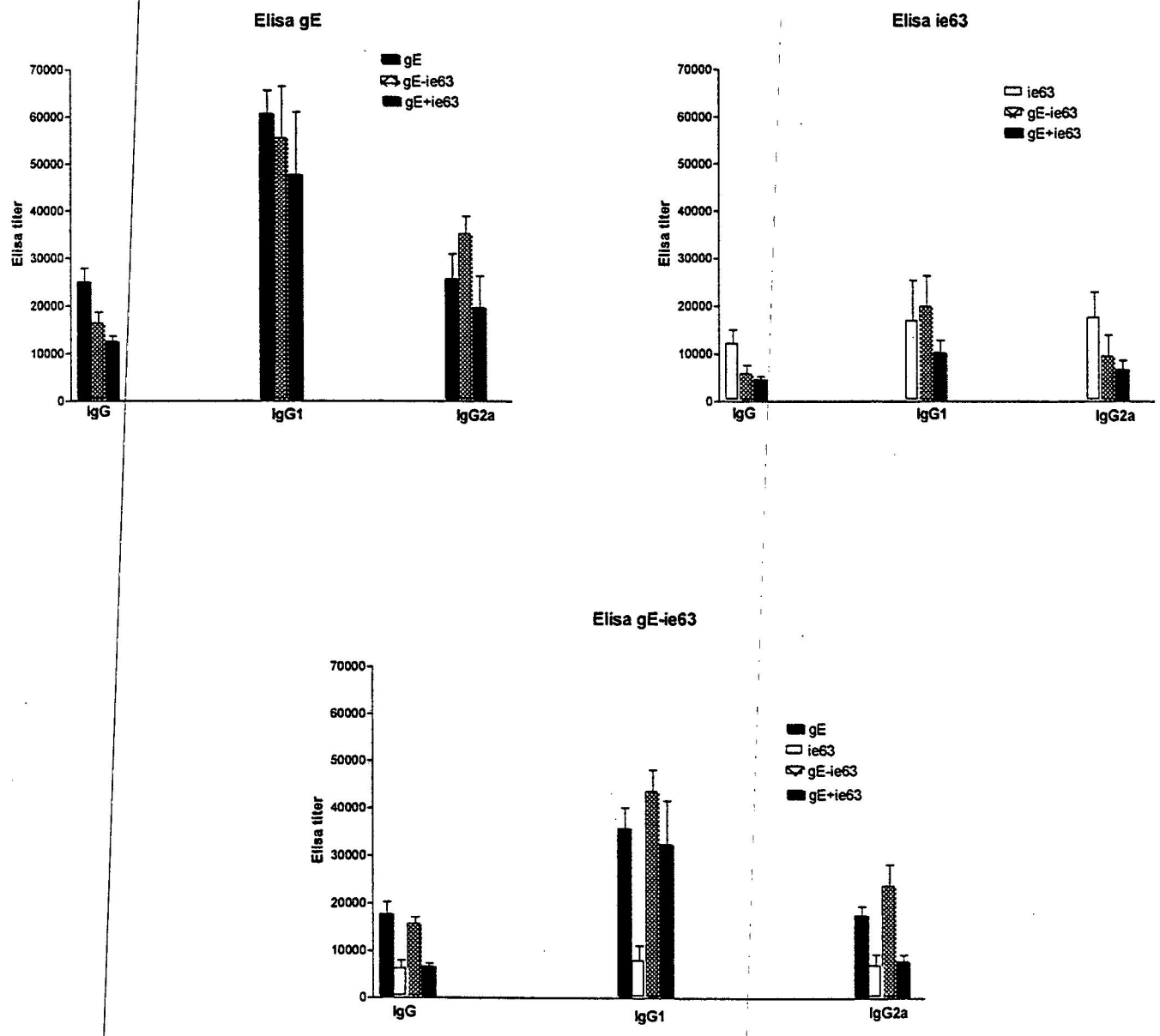


Figure 9

11/12

107
AJ
10/2

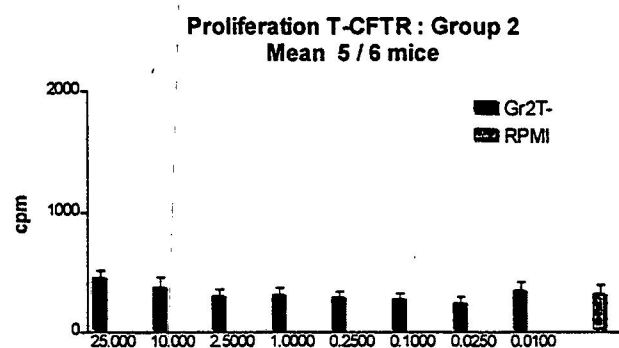
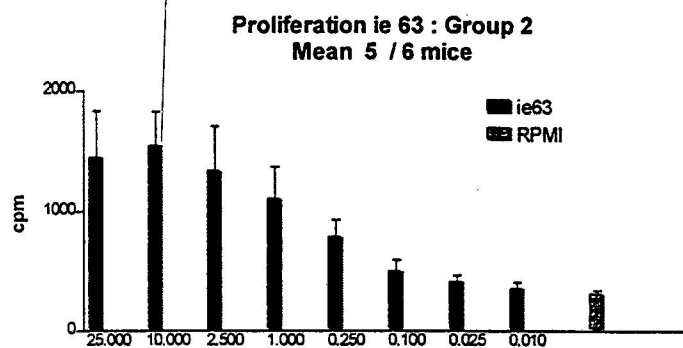


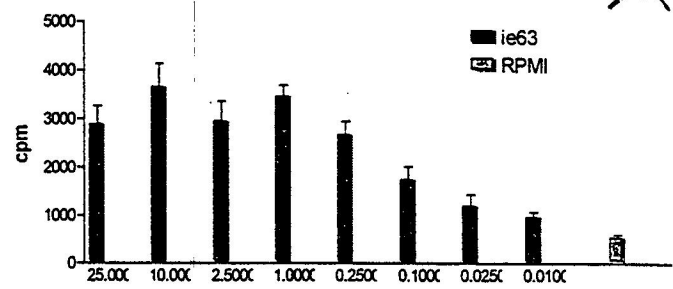
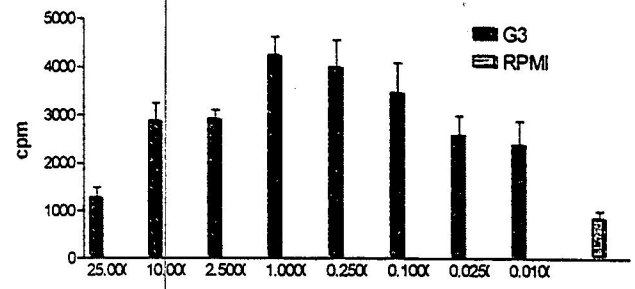
Figure 10

12/
12

108 

Proliferation gE-ie63 : Group 3
Mean 4 / 5 mice

Proliferation ie63 : Group 3
Mean 5 / 6 mice



Proliferation T-CHO : Group 3
Mean 5 / 6 mice

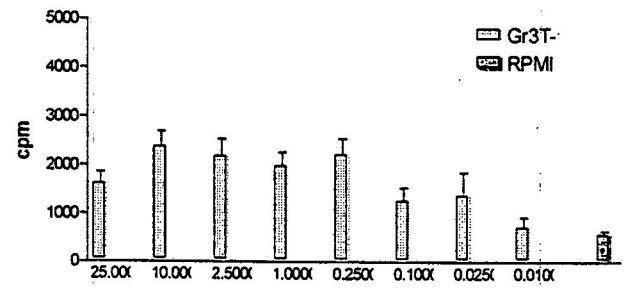


Figure 11

109 AG
III

LEGALIZACIONES

OFICINA DE PATENTES

Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

El suscrito, un funcionario debidamente autorizado conforme a los Artículos 74(1) y (4) de la Ley de Desreglamentación y Contratación de 1994 para firmar y expedir certificados en nombre del Contralor General, certifico que la copia adjunta es una copia correcta de los documentos archivados originalmente con respecto a la solicitud de patente que allí se identifica.

De acuerdo con las Reglamentaciones de Patentes de 1982 (Re-registro de Compañías), si una compañía nombrada en este certificado y en los documentos adjuntos se ha vuelto a registrar según la Ley de Compañías de 1980 con el mismo nombre con el que fue registrada inmediatamente antes del re-registro, salvo la sustitución o inclusión como la última parte del nombre de las palabras "sociedad anónima" o su equivalente en galés, las referencias al nombre de la compañía y sus documentos adjuntos serán tratadas como referencias al nombre con el cual fue registrada.

De acuerdo con las reglas, las palabras "sociedad anónima" (o en inglés *public limited company*) pueden ser reemplazadas por las letras p.l.c, plc. P.L.C ó PLC.

El re-registro bajo la Ley de Compañías no constituye una nueva entidad legal sino simplemente somete a la compañía a ciertas normas de ley adicionales.

(FIRMA) ANDREW CRESSEY

FECHA: 22 de diciembre de 1999

(SELLO DE LACRE)

110
42

OFICINA DE PATENTES

Referencia No. **FB/B45165** No. **9901254.4** 20 de enero de 1999

OFICINA DE PATENTES: Solicitud de adjudicación de una Patente.

Forma 1/77

Ley de Patentes de 1977

1. TITULO DEL INVENTO: "COMPUESTOS NOVEDOSOS"

2. INFORMACIÓN DEL SOLICITANTE:

2a. Nombre: SmithKline Beecham Biologicals S.A.

Pais: Bélgica

2b N/A

2c Dirección: Rue de l'Institut 89
B-1330 Rixensart
Bélgica

Código Postal:

PAIS. BELGICA

ADP No. 5800974002

2d Segundo Solicitante:

N/A

3. Ha nombrado Ud. a alguien que le represente en su solicitud? SI

Nombre del Agente: A W R TYRRELL

SmithKline Beecham

Corporate Intellectual Property

Two New Horizons Court,

Brentford, Middlesex

Código postal TW8 9EP

No. ADP 6684161002

4. Número de Referencia: **FB/B45165**
5. Solicita Ud. que esta solicitud sea tratada como si hubiera sido radicada en la fecha de presentación de una solicitud anterior: NO
6. No aplica
7. Es usted el único inventor o inventor conjunto? NO
8. Cuántas hojas describen esta solicitud? 18 - Reivindicaciones: 3-

Resumen: 1 - - Dibujos - 12 -

9. Solicitud: Solicitamos la adjudicación de una patente teniendo en cuenta esta solicitud.

(FIRMA) A W R TYRRELL

Fecha: 15/01/99

Abogado de Patentes

Favor devolver la forma diligenciada y sus anexos y duplicados, junto con su valor a:

The Comptroller
The Patent Office
Cardiff Road
Newport
Gwent
NP9 1RH

ó

The Comptroller
The Patent Office
25 Southampton Buildings
London
WC2A 1AY

VACUNAS

112

AA
114

REIVINDICACIONES:

1. Una proteína de fusión que comprende:
 - (1) una proteína gE del virus Varicella Zoster o un fragmento inmunológicamente activo de la misma fusionado a:
 - (2) una proteína diferente del virus Varicella Zoster o a un fragmento inmunológicamente activo de la misma
2. Una proteína de fusión según la reivindicación 1, que comprende:
 - 1) una proteína gE del virus Varicella Zoster o un fragmento inmunológicamente activo de la misma fusionado a:
 - 2) una proteína del virus Varicella Zoster IE63 o a un fragmento inmunológicamente activo de la misma.
3. Un ADN o ARN recombinante que codifica una proteína de fusión según cualquiera de las reivindicaciones anteriores.
4. Un vector de expresión o un microorganismo vivo recombinante que comprende ADN o ARN recombinante según la reivindicación 3.
5. Una célula huésped transformada con ácido nucleico según la reivindicación 3, o con un vector según la reivindicación 4.
6. Una composición de vacuna que comprende una proteína de fusión según la reivindicación 1 o 2 y un excipiente farmacéuticamente aceptable.

- 113 
7. Una composición de vacuna que comprende ácido nucléico y que codifica la proteína de fusión de la reivindicación 1 o la reivindicación 2 y un excipiente farmacéuticamente aceptable.
 8. Una proteína de fusión según la reivindicación 1 o la reivindicación 2 para uso en medicina.
 9. Un proceso para la producción de una proteína de fusión según la reivindicación 1 o la reivindicación 2, cuyo proceso comprende expresar ADN recombinante que codifica dicha proteína en una célula anfitriona y recuperar la proteína.
 10. El uso de una proteína de fusión según la reivindicación 1 ó 2 para la fabricación de un medicamento para la prevención o alivio de Varicella o Zoster.
 11. Una composición de vacuna según la reivindicación 6 ó 7, que comprende adicionalmente otros antígenos VZV.
 12. Una composición de vacuna según la reivindicación 11, en donde la otra proteína del Virus Varicella Zoster es seleccionada del grupo gE, gB, gH, gI, gCX o IE62 o de un derivado inmunológicamente funcional de la misma.
 13. Una composición de vacuna según la reivindicación 6, 7, 11 ó 12, que adicionalmente comprende un adyuvante.
 14. Una composición de vacuna según la reivindicación 13, en donde el adyuvante induce preferencialmente una respuesta TH1.
 15. Una composición de vacuna según la reivindicación 13, en donde el adyuvante es seleccionado del grupo de adyuvantes que comprenden; 3D-MPL, QS21, una mezcla de QS21 y colesterol, un oligonucleótido CpG,

114 ~~74~~
~~110~~

hidróxido de aluminio, fosfato de aluminio y tocoferol, y agua en emulsión de aceite o una combinación de dos o más de dichos adyuvantes.

16. Un método para tratar a un paciente que sufra de o que sea susceptible a infección o recurrencia del Virus Varicella Zoster, que comprende administrar a un paciente una cantidad segura y efectiva de una composición de vacuna según cualquiera de las reivindicaciones 6, 7, 11, 12, 13, 14 ó 15.
17. Un método para producir una composición de vacuna según cualquiera de las reivindicaciones 6, 7, 11 ó 12, que comprende mezclar dicha proteína de fusión o ácido nucleico con un excipiente farmacéuticamente aceptable.
18. Un método según la reivindicación 17, en donde el excipiente comprende un adyuvante según las reivindicaciones 13 a 15.

2do. EXAMEN DE FORMA PATENTE DE INVENCION

115

Folio 120

Radicación no 00-2923

Concepto Técnico no 1461

Clasificación Internacional de Patentes: A61K 39/29

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486 se determinó que esta solicitud de **Patente de Invención**:

- SI ☒ NO ☐ Contiene un resumen de la invención (Art.26-e).
- SI ☒ NO ☐ Contiene el título o nombre de la invención (Art.27-d).
- SI ☒ NO ☐ Contiene la descripción de la invención (Art.26-b).
- SI ☒ NO ☐ NO ES APLICABLE ☐ Contiene los dibujos necesarios para comprender la invención (Art.26-d)
- SI ☒ NO ☐ Contiene una o más reivindicaciones. (Art.26-c).
- SI ☐ NO ☐ NO ES APLICABLE ☒ Han sido desarrollados los productos y/o procedimientos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen. (Art. 26- h).
- SI ☐ NO ☐ NO ES APLICABLE ☒ Los productos y/o procedimientos han sido obtenidos o desarrollados a partir de los conocimientos tradicionales de las comunidades indígenas, afroamericanas, o locales de los países miembros, de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes.(Art.26-i).
- SI ☐ NO ☒ La invención se refiere a un producto relativo a un material biológico. (Art.26-J).
- SI ☒ NO ☐ La prioridad coincide con la materia reivindicada.
- SI ☒ NO ☐ **CUMPLE LOS REQUISITOS TÉCNICOS**

OBSERVACIONES Y OTROS: SI ☐ NO ☒ ver hoja(s) anexa(s)

Nuevo título folio 83.

EXAMINADOR TÉCNICO
202016

Bogotá D. C., 2002-09-30

Folio 121

116
416
2002-10-07

2020
Bogotá DC

Doctor(a)
GERMÁN CASTILLO GRAU
Apoderado(a)

REFERENCIA:	Radicación No.	00-2923
	Trámite	002
	Evento	001
	Actuación	416
	Oficio No.	1971
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la misma Decisión.

Cúmplase

Dado en Bogotá DC, a los

07 OCT. 2002


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

Bogotá, DC, Dy 1-03 El extracto de esta solicitud fue publicado en el número 524 de la Gaceta de Propiedad Industrial, de fecha 31-01-03.
El término hábil señalado por la ley expiró el 30-04-03 y SI —
NO X se presentaron oposiciones por parte de terceros.


202016

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: 0-2923

EDICTO No. 13589

LA SECRETARÍA GENERAL AD-HOC HACE SABER:

Que esta Superintendencia profirió **Resolución No. 22805 de 19-AGO-2003**. Que el contenido de la parte resolutive es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

ARTICULO PRIMERO: Declarar abandonada la solicitud de privilegio de patente de invención contenida en el expediente de la referencia.

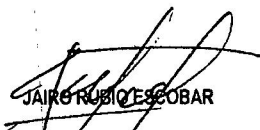
ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor Germán Castillo Grau, o a quien haga sus veces, quien actúa como apoderado de Smithkline Beecham Biologicals S.A., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE

Dada en Bogotá, D.C., a los

19 AGO. 2003

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,


JAIRO RUBIO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc 02-SEP-2003  Este edicto se desfija hoy 15-SEP-2003 a las cinco (5:00 p.m.) _____
Secretaría General Ad-Hoc
EDICTO No. 13589