Factors that Influence the Immunogenicity of Human Recombinant Interferon Alpha-2b in Mice

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ABSTRACT

Several factors that can influence the immunogenicity of a recombinant human IFNα-2b formulation (Heberon Alfa R) were evaluated in mice. They were: frequency and route of administration, presence of human serum albumin (HSA) in the formulation, and the major histocompatibility complex (MHC) genotype. The presence of anti-IFNα-2b antibodies in the sera was detected by a sandwich-type ELISA system. Mice immunized with 100 000 IU of IFNα-2b three times a week produced high anti-IFN antibody titers earlier than those injected with 300 000 IU once a week. Titers were higher when the IFN was administered intraperitoneally as compared to the subcutaneous, intravenous, and intramuscular routes. On the other hand, the anti-IFN antibody titers were higher, but not statistically significant, when HSA was present in the formulation. Among the different mouse strains tested, higher titers were obtained in the order BALB/c >> B6D2F1 > NMRI >> C57BL/6. No anti-IFN antibodies were detected in the latter. This is the first study where the influence of these factors on the immunogenicity of the Cuban recombinant interferon is reported.

Keywords: administration, albumin, anti-IFN antibodies, frequency, IFNα-2b, immunogenicity, major histocompatibility complex, routes

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RESUMEN

Factores que influyen en la inmunogenicidad del IFN alfa-2b humano recombinante en ratones. Se analizó la influencia ejercida por varios factores en la inmunogenicidad del IFNα-2b humano recombinante (Heberon Alfa R) en ratones. Éstos fueron: frecuencia y vía de administración, presencia de albúmina sérica humana (HSA) como excipiente en la formulación y genotipo del complejo mayor de histocompatibilidad (MHC). La presencia de anticuerpos anti IFNα-2b en suero se detectó por un sistema ELISA tipo "*sandwich*". Los ratones inmunizados con 100 000 UI de IFNα-2b tres veces por semana produjeron títulos elevados de anticuerpos más rápidamente que los inyectados con 300 000 UI una vez por semana. Los títulos fueron mayores cuando el IFN se administró por vía intraperitoneal, comparados con la subcutánea, intravenosa e intramuscular. Por otra parte, los títulos fueron mayores, aunque sin significación estadística, cuando la HSA estuvo presente en la formulación. Entre las diferentes líneas de ratón evaluadas, el orden de los títulos fue el siguiente: BALB/c >> B6D2F1 > NMRI >> C57BL/6. La línea C57BL/6 no produjo anticuerpos anti-IFN. Este es el primer reporte sobre la influencia de estos factores sobre la inmunogenicidad del IFN recombinante cubano.

Palabras claves: administración, albúmina, anticuerpos anti-IFN, complejo mayor de histocompatibilidad, frecuencia, IFNα-2b, inmunogenicidad, vías

*I*ntroduction

The interferons (IFNs) were among the first cytokines to be cloned and expressed in microorganisms. They have been the most widely investigated cytokines as well, both at the molecular level and in their clinical applications. The amino acid sequences of human recombinant IFNs are similar to those of their natural homologous species, but they can differ in glycosylation patterns, other post-transcriptional changes and small differences in their three-dimensional structures. These factors can render recombinant IFN immunogenic in patients receiving them.

Vallbracht *et al.* [1] were the first to report the presence of antibodies to IFN in patients. Thereafter, the induction of antibodies has been reported in various clinical studies with different commercial preparations of human recombinant IFN in patients with several diseases [2, 3]. The incidence of antibodies varies greatly: in some studies antibodies are not found at all, while in others it reaches 80%.

The analysis of IFN immunogenicity and its formulations is complex and requires continuous research since many questions on the causes and factors involved are not yet clarified. It is not easy to perform controlled studies in humans with this purpose. Therefore, it is necessary to carry out experiments on animal models that could permit predictions in the clinical setting.

In this report, the influence of some factors on the immunogenicity of an IFNα-2b commercial preparation (Heberon Alfa R) in immunocompetent mice is shown. The frequency of IFNα-2b injections, routes of administration (intramuscular, subcutaneous, intravenous, and intraperitoneal), the presence of human serum albumin (HSA) as ingredient in the formulation, as well as different strains of mice for testing the influence of the major histocompatibility complex (MHC) genotype were studied. A simple immunoassay was developed for the anti-IFN antibodies detection in mouse serum and the verification of their specificity.

*M*aterials and Methods

Animals

Male BALB/c, NMRI, B6D2F1 and C57BL/6 mice from the National Center for the Production of Labo-

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1. Vallbracht A, Treuner J, Flehmig B, Joester KE, Niethammer D. Interferon-neutralizing antibodies in a patient treated with human fibroblast interferon. Nature 1981;289: 496–7.

2. Antonelli G, Simeoni E, Currenti M, Depisa F, Colizzi V, Pistello M, Dianzani F. Interferon antibodies in patients with infectious diseases. Biotherapy 1997;10: 7–14.

3. Öberg K, Alm G. The incidence and clinical significance of antibodies to interferon-a in patients with solid tumors. Biotherapy 1997;10:1–5.

ratory Animals (CENPALAB, Havana) were used. They were kept under controlled conditions of temperature (19 ± 2 ºC) and humidity (55-65%). Food and water intake were *ad libitum*.

Interferon

Heberon Alfa R (Heber Biotech S.A., Havana) was available as a lyophilized powder containing IFNα-2b (3×10^6 IU), 1.5 mg HSA, 10 mg dextran, 5 mg manitol, 7 mg NaCl, 11.4 mg $Na_2HPO_4.2H_2O$, and 6 mg $NaH_2PO_4.2H_2O$ per vial. It was reconstituted with 0.9% NaCl and diluted to the desired concentrations. In one of the experiments a liquid formulation without albumin was used. It contained, per vial, IFNα-2b (10×10^6 IU), 10 mg benzyl alcohol, 0.2 mg Tween 80, 4.67 mg NaCl, 14.41 mg $Na_2HPO_4.2H_2O$, 2.96 mg $NaH_2PO_4.2H_2O$ and distilled water to complete 1 mL. For the ELISA, buffered human recombinant IFNα-2b (see below) was used.

Experimental designs

All the immunization schedules lasted 4 weeks. A previous experiment was carried out to learn the dose at which the anti-IFN antibodies are generated in mice, which was used in the study (data not shown). In all experiments mice were distributed at random among the experimental groups. Each group of animals was divided into four parts. The mice were slaughtered and bled at the end of each week of the treatment to verify the development of anti-IFN antibody titers. The sera were stored at - 20 ºC until their analyses.

The effect of the frequency of administration on IFNα-2b immunogenicity was studied using 80 BALB/c mice distributed into two groups. The first group received 300 000 IU of IFNα-2b intraperitoneally once a week; the second was immunized with 100 000 IU three times a week through the same route.

To study the effect of the administration route, 160 BALB/c mice were distributed into four groups, that were: intraperitoneal (i.p.), subcutaneous (s.c.), intramuscular (i.m.) and intravenous (i.v.). Mice were injected three times a week with 100 000 IU of IFNα-2b.

The relationship between the presence of albumin in the IFNα-2b formulation and antibody induction was evaluated. Forty BALB/c mice were i.p.-injected three times a week with 100 000 IU of the IFNα-2b preparation containing albumin by the intraperitoneal route and another forty received the albumin-free formulation at the same dosage and route.

Different strains of mice were tested to learn the influence of the MHC genotype in IFN immunogenicity. NMRI, B6D2F1 and C57BL/6 mice (40 animals each) were i.p.-immunized three times a week, intraperitoneally, with 100 000 IU of IFNα-2b.

Determination of anti-human IFNα antibodies in mice sera

A double antigen ELISA was developed. Positive controls were the serum of a patient with neutralizing antibodies at the beginning and later, a pool of sera from mice with high anti-IFN titer. The sera of non-immunized BALB/c mice were used as the negative controls. The ninety-six well polystyrene plates (Costar, Cambridge, MA) were coated with 100 mL/well of IFNα-2b (20 μg/mL) in 0.1 mol/L Na_2CO_3 - $NaHCO_3$ pH 9,6 for 3 h at 37 ºC. They were washed once with 0.05 % Tween 20 (Sigma, USA) and incubated with the samples or controls (100 μL/well) for 2 h at 37 ºC. The samples were diluted in 2% skim milk (Oxoid) in PBS, pH 7.2 - 7.4 (assay buffer). After 4 washings, an IFN-peroxidase conjugate (Immunotechnology and Diagnosis Division, CIGB) was added (100 μL/well) and incubated for 30 min at 37 ºC. After 6 washings the reaction was developed for 15 min at room temperature with 5.5 mg of *o*-phenylendiamine (Merck, Germany) and 5.5 mL of 0.015 % H_2O_2 (Caledon) in 11 mL of citrate-phosphate pH 5.0 (100 μL/well). Finally, the reaction was stopped with 50 μL/well of 2 M H_2SO_4. The absorption was read at 492 nm in a plate reader (SUMA 031, Tecnosuma, Havana).

Eight dilutions: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560 were tested. The sera with extinction values above 3 standard deviations over the average of the negative controls in each plate (cut off) were considered as positive antibody responses. The antibody titer was the inverse of the highest positive dilution. Simultaneously, to test specificity, the samples were pre-incubated with 100 μg/mL IFNα-2b for 1 h at 37 ºC.

Statistical analysis

The results of the anti-IFN antibody titers obtained under the experimental conditions were compared to the pool of results obtained with BALB/c mice, i.p.-immunized with 100 000 IU of IFNα-2b three times a week. This group of mice was present in all the experiments except for the MHC genotype test.

For each evaluation time, an analysis of variance (ANOVA) or Kruskall–Wallis test was applied with the logarithms of the titers obtained with a Bonferrony adjustment, depending on the assumption of normal distribution and variance homogeneity. When those differences were statistically significant, multiple comparisons tests (Tuckey or Duncan) were carried out.

Results

Figure 1 shows that IFNα-2b three times a week at a low dose yielded higher antibody titers faster than a high dose once a week ($p = 0.010$).

Starting from the second week of immunization, the intraperitoneal route showed the highest antibody titers, while the other routes behaved similarly during the whole experimental procedure (Figure 2). This difference was statistically significant ($p < 0.05$).

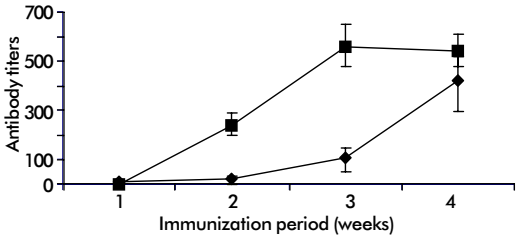

Figure 1. Antibody titers against IFNα-2b in BALB/c mice immunized by the intraperitoneal route with 300 000 IU once a week (-◆-) or 100 000 IU three times a week (-■-), during four weeks ($p < 0,05$).

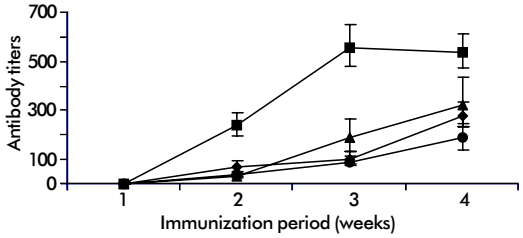

Figure 2. Antibody titers against IFNα-2b in BALB/c mice immunized with 100 000 IU three times a week, through different administration routes, during four weeks, -◆-i.m., -■-i.p., -▲-i.v., -●-s.c. ($p < 0.05$).

No significant differences were detected in the induction of antibodies between the formulations containing or not HSA (Figure 3), although at the third week of immunization there was a tendency toward higher titers in mice that received the HSA-containing product ($p = 0.09$).

Figure 4 shows that BALB/c mice produced much higher anti-IFN antibody titers with respect to the other strains. C57BL/6 mice did not produce antibodies at all. The order of titers was: BALB/c >> B6D2F1 > NMRI >> C57BL/6. All differences were statistically significant ($p < 0.001$ in all cases), except between NMRI and B6D2F1.

Antibodies were not detected after the first week of immunization in any of the experiments. The specificity of the immunoassay was confirmed (Figure 5). The absorbance values dropped completely after preincubation with IFNα-2b (100 μg/mL) when the values are compared with those for the positive samples at the same dilution.

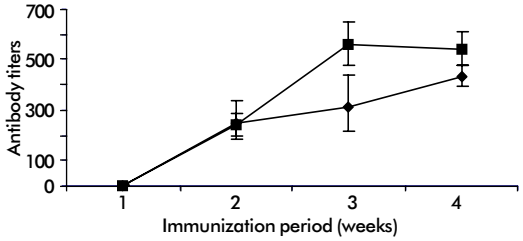

Figure 3. Antibody titers against IFNá-2b in BALB/c mice after immunization with 100 000 IU three times a week, by the intraperitoneal route, with a commercial formulation that contains HSA (Heberon Alfa R, lyophilized) (-■-) or a liquid formulation without albumin (-◆-).

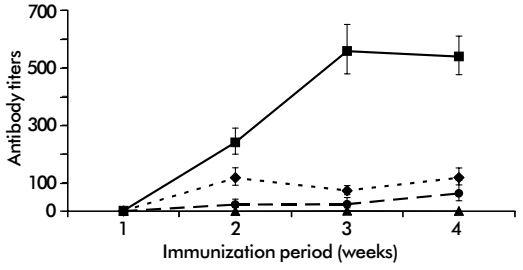

Figure 4. Antibody titers against IFNα-2b in different strains of mice immunized with 100 000 IU three times a week, through the intraperitoneal route, during four weeks. BALB/c mice data are the average of those corresponding to Figures 1, 2 and 3. -■-BALB/c, -◆- B6D2F1, -●- NMRI, -▲- C57BL/6. Except between F1 and NMRI mice. the differences were significant in all cases ($p < 0.001$).

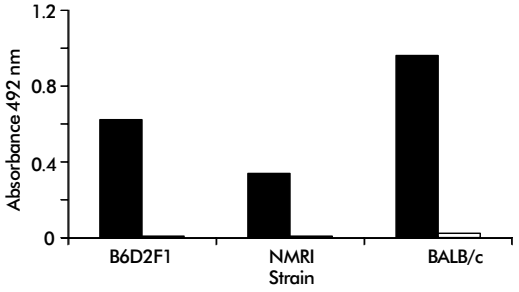

Figure 5. Specificity test for anti-IFN antibodies in mice sera. The average values of the positive samples from one of the experiments at the 1:20 dilution with (-□-) or without (-■-) pre-incubation with 100 μg/mL of IFNα-2b are represented.

Discussion

The Center for Genetic Engineering and Biotechnology (CIGB, Havana) produces Alfa R, a human recombinant IFNα-2b expressed in bacteria *(Escherichia coli)* with a high purity. This product has shown its efficacy in the treatment of both viral and oncological affections in several clinical trials [4-7] Nevertheless, neutralizing antibodies for IFNα-2b have been detected in 3.2 % of all the patients treated with Heberon Alfa R [8, article in preparation]. These neutralizing antibodies may be of practical importance since they could be a cause of the lack of response to the treatment with this molecule. There are studies where tumor relapses in some patients have coincided with the development of neutralizing antibodies to IFNα-2b [9-12]. In some cases, the response can be recovered if therapy is switched to natural IFN [13]. On the other hand, certain authors have not found clinical implications due to the generation of these antibodies [14, 15]. It is possible that, in these cases, the affinity of the IFN receptors for the IFN was higher than the antibodies present in serum. Alternatively, patients could have responded before developing the neutralizing antibodies. In cases of prolonged treatment or high doses, the formation of antibodies constitutes a potential problem [16]. These antibodies could also trigger the autoimmune processes [17].

This immune response depends on a great variety of protein-, formulation- and subject-dependant factors. Antibodies induced by any of the commonly used recombinant IFNα-2's can recognize all of them by both ELISA and the neutralization of the antiviral activity assay. Some contaminating forms with immunogenic properties have been detected [18]. Differences in the manufacturing and purification procedures can also influence the antigenicity reported for the commercial formulations of IFN [19]. On the other hand, for many years all IFN formulations have contained HSA as the stabilizer, but immunogenic reactions could be expected with that product [20] as well. Since the albumin can interact with other proteins [21, 22], IFN–HSA aggregates can form [23], which increases its molecular weight, and therefore immunogenicity. For storage, a low tem-

4. Arús E, Infante M, Padrón GJ, Morales MG, Grá B, López-Saura P. Interferón alfa-2b recombinante en hepatitis C crónica: resultados del tratamiento y determinación de anticuerpos anti-interferón. Biotecnología Aplicada 1997;14:242–7.

5. Martínez E, Miranda N, Garmendía G, *et al*. Succesful treatment of pediatric hemangioma with interferon alpha-2b. J Interferon and Cytokine Res 1997;17 Suppl 2:108.

6. Collazo S, Moreno E, García Y, *et al*. Intralesional injection of recombinant interferon alpha 2b in Basal cell carcinoma. Avances en Biotecnología Moderna 1999;5:O48.

7. Díaz de la Rocha A, Álvarez MJ, Sagaró-Delgado B, Guillama E, López-Saura P. Tratamiento combinado tópico y sistémico de condilomas acuminados con interferón alfa-2b recombinante o factor de transferencia. Ensayo clínico aleatorizado, a doble ciegas, controlado con placebo. Biotecnología Aplicada 1997; 14:248–52.

8. González R, Ferrero J, Morales MG, Aguilera A, López P. Inmunogenicidad del interferon alfa-2b recombinante (Heberon Alfa R). Detección de anticuerpos mediante un ensayo inmunoenzimático y neutralización de actividad antiviral. Biotecnología Aplicada 1998;15:71–6.

9. Davis-Daneshfar A, Böni R, Von Wussow P, Joller H, Burg G, Dummer R. Adjuvant immunotherapy in malignant melanoma. Impact of antibody formation against interferon-α on immunoparameters *in vivo*. J Immunotherapy 1997;20:208–13.

10. Öberg K, Alm G, Magnusson A, *et al*. Treatment of malignant carcinoid tumors with recombinant interferon-α2b (Intron A): development of neutralizing interferon antibodies and possible loss of antitumor activity. J Natl Cancer Inst 1989;81:531–5.

11. Russo D, Candoni A, Grattoni R. Clinical experience of antibodies to interferon-a during treatment of chronic myeloid leukemia. J Interferon and Cytokine Res 1997;17Suppl 1:47–9.

12. McKenna RM, Öberg K. Antibodies to interferon-α in treated cancer patients. Incidence and significance. J Interferon and Cytokine Res 1997;17Suppl 1:141–3.

13. Antonelli G. *In vivo* development of antibody to interferons. An update to 1996. J Interferon and Cytokine Res 1997;17Suppl 1:39–46.

14. Spiegel RJ. The alpha interferons: clinical overview. Semin Oncol 1987;14: 1–12.

15. Steis RG, Smith JW, Urba WJ, *et al*. Resistance to recombinant interferon alpha-2a in hairy cell leukemia associated with neutralizing anti-interferon antibodies. New Engl J Med 1988;318:1409–13.

16 . Dianzani F, Pestka S. Introduction. J Interferon Cytokine Res 1997;17 Suppl 1:S3.

17. Lensch E, Faust J, Nix W.A, Wandel E. Myasthenia gravis after interferon-alpha treatment. Muscle and Nerve 1996;927–8.

18. Hochuli E. Interferon immunogenecity: technical evaluation of interferon-α2a. J Interferon and Cytokine Research 1997; 17Suppl 1:15–21.

perature (2–8 ºC) and short periods are preferred, since antibodies have been observed in recombinant IFNα-2a vials stored at room temperature [18].

The characteristics of the disease under treatment affects the generation of antibodies as well. A high incidence has been reported in oncological [24] and viral [25] diseases. Individual characteristics may also be implicated. In a previous report [26] there were differences in the antibody production in several strains of mice. The study of the frequency of administration has revealed that the more frequently the patient receives IFN, the higher the possibility to develop antibodies [27], which has also been observed in mice. Some routes of administration have been shown to be more immunogenic than others [26]. Some articles comment that the higher the dose and the duration of the treatment with recombinant IFN, the greater the possibility of developing antibodies [27].

In this study, the mice immunized more frequently with a low dose developed the earliest and highest antibody titers. Apparently, the antibody response depends on the availability of antigen and not on the immunization dose, since for any administration route IFNs have very short half-life periods in the body fluids. This permits a longer recognition time for the antigen when a more repetitive presentation to the immune cells is achieved. Only after four weeks of immunization are the antibody titers similar.

The intraperitoneal route of administration was the most immunogenic. The peritoneum is a very irrigated area, with abundant drainage. IFN concentration in this cavity could be much higher than in the blood, which propitiates a lingering stay in the peritoneum and therefore a slow clearance, with a possible immunoadjuvant effect [28]. This fact could explain the difference with the subcutaneous route, which also has a high lymphatic irrigation. Besides, since the peritoneum is a cavity, a higher interaction between the immunogen and the lymphocyte population could occur. Since Heberon Alfa R had a similar immunogenicity by the intramuscular and subcutaneous routes their interchangeably for treatments in patients could be assumed, at least in regard to this aspect. In contrast, Palleroni *et al.* [26] obtained higher titers for intraperitoneal and subcutaneous routes using IFNα-2a in BALB/c mice whereas the intramuscular and intravenous routes did not produce antibodies. We consider that the IFN subtype could have influenced this. In fact, IFNα-2a has been shown to be immunogenic. Nevertheless, further studies are necessary to clarify the possible differences in immunogenicity of IFNα when administered by the subcutaneous, intravenous or intramuscular routes.

IFNα-2b formulations did not show statistically significant differences on antibody production, although the formulation that contains albumin yielded a higher titer at three weeks. The formation of more immunogenic IFN–HSA aggregates depends closely on characteristics such as pH and storage temperature and time, which needs to be studied separately and thoroughly. There is another report of similar HSA–IFN aggregate formation between INTRON A (Schering Plough) and Roferon A (Hoffman la Roche) formulations, despite important differences in their albumin content [23].

According to our findings, studies in different mouse strains reveal that there is a genetic regulation in the immune response (MHC) to human recombinant IFNα-2b. For instance, BALB/c mice (haplotype H-2^d) developed the highest antibody titers, while C57BL/6 (haplotype H-2^b) did not produce detectable titers. Hybrid B6D2F1 (both alleles) and NMRI mice had a similar behavior. The entire MHC genotype of the NMRI mice has not yet been characterized, so it is very difficult to predict a conduct with this immunization. However, their antibody production seems to be logical, since within such a large variety of genes the "high-producing" alleles may be present.

The immunoassay performed to measure antibody titers was satisfactory in terms of sensitivity, precision and high specificity. Absorbance values of the positive samples were substantially reduced by preincubation with soluble IFNα-2b. Since there are no reports on ELISA systems for the determination of antibodies against recombinant IFNα in mice sera, this method can be applied in future investigations. Similar sandwich-type ELISAs refer only to the determination on human serum [29].

It was shown that the factors tested can influence IFN immunogenicity. It is necessary to speculate on the possible extrapolation of these results to patients that receive recombinant IFNα-2b. There are few studies in humans with observations on these factors (18, 27). Due to ethical and practical reasons it is difficult to carry out such studies under controlled conditions. Besides, antibody titers should be much lower in humans than in rodents due to the poor molecular homology between IFN's from both sources [30]. Although there are no data for MHC in humans, it is conceivable that the treated patients' MHC genotype can influence the incidence and intensity of antibody response, since some natural autoantibodies appear in autoimmune diseases [31]. The effect of albumin content on IFNα-2b immunogenicity is not reported in humans or animals.

Although the extrapolation to the clinical setting is limited, as explained above, this study contributes to the characterization of the biological properties of Cuban recombinant IFN. The identification of the factors that affect its immunogenicity will contribute to envisage different therapeutic regimes and eventually confirm them in humans through thorough observations in treated patients.

19. Spiegel RJ, Jacobs SL, Treuhaft MW. Anti-interferon antibodies to interferon-α2b: results of comparative assays and clinical perspective. J Interferon Research 1989;9:S17–4.

20. Gales B, Erstead B. Adverse reactions to human serum albumin. Ann Pharmacother 1993;27:87–94.

21. Kumarasamy R, Bauch J, Kopcha D, Patel S, McGonigle. An enzyme-linked immunosorbent assay (ELISA) for quantitation of adducts of granulocyte-macrophague colony stimulating factor (GM-CSF) and human serum albumin (HSA) in stressed solution mixtures. E Pharm Res 1994;11:365–71.

22. Yoshioka S, Aso Y, Izutzu K, Terao TJ. Aggregates formed during storage of beta-galactosidase in solution and in the freeze-dried state. Pharm Res 1993;10:687–91.

23. Braun A, Alzenz J. Development and use of enzyme-linked immunosorbent assays (ELISA) for the detection of protein aggregates in interferon-alfa (IFN-α) formulations. Pharmaceutical Research 1997;14:10:1394–400.

24. Prümmer O. Interferon-alpha antibodies in patients with renal cell carcinoma treated with recombinant interferon-alpha-2a in an adjuvant multicenter trial. Cancer 1993;71:1828–34.

25. Bonetti P, Diodati G, Drago C, *et al*. Interferon antibodies in patients with chronic hepatitis C virus infection treated with recombinant interferon alpha-2a. Hepatol 1994;20:416–20.

26. Palleroni AV, Aglione A, Labow M, Brunda MJ, Pestka S, Sinigaglia F, Garotta G, Alzenz J, Braun A. Interferon immunogenicity: Preclinical evaluation of interferon-a2a. J Interferon and Cytokine Research 1997;17Suppl 1:23–7.

27. Itri LM, Sherman MI, Palleroni AV, Evans ML, Tran L, Campion M, Chizzonite R. Incidence and clinical significance of neutralizing antibodies in patients receiving recombinant interferon-alpha 2a. J Interferon Res 1989;9:S9.

28. Bocci V. Pharmacokinetics of interferons and routes of administration. In: Baron S, Coppenhaver DH, Dianzani F, *et al.*, editors. Interferons: principles and medical applications. Texas: The University of Texas Medical Branch at Galveston; 1992. p.417–25.

29. Von Wussow P, Jakschies D, Freund M, Deicher H. Humoral response to recombinant interferon-a2b in patients receiving recombinant interferon α2b therapy. J Interferon Res 1989;9:S25.

30. Raj NBK, Israeli R, Kelley KA, Leach SJ, Minasian E, Sikaris K, *et al*. Synthesis, antiviral activity, and conformational characterization of mouse-human α-interferon hybrids. J Biological Chemistry 1988;263:8943–52.

31. Meager A. Natural autoantibodies to interferons. J Interferon and Cytokine Research 1997;17Suppl 1:51–3.

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