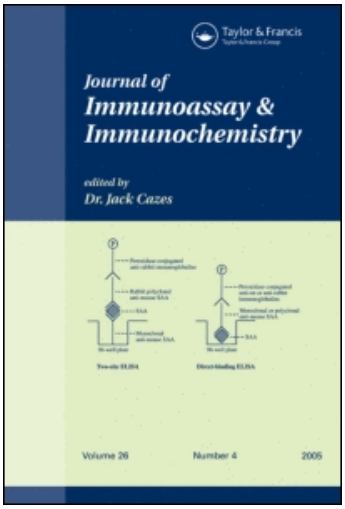

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Complement-Mediated Inactivation of Interferon-Gamma in Elisa Systems

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COMPLEMENT-MEDIATED INACTIVATION OF INTERFERON-GAMMA IN ELISA SYSTEMS

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ABSTRACT

The recovery of a predetermined amount of interferon-gamma (IFN-γ) added to normal serum was studied in two independent sandwich ELISA systems specific for rat and human IFN-γ. In both assays the ELISA activity was rapidly lost in fresh but not in heat-inactivated (30', 56°C) serum. Ninety percent of the initial activity had disappeared within 30 minutes upon incubation at 37°C. Serum-mediated inhibition was not species-specific as the ELISA activity of rat IFN-γ diminished equally well in rat and human sera. Inhibition was critically dependent on the isotype of the solid-phase monoclonal antibody (mAb) used in the ELISA systems. IgG_1 and IgG_{2a} mAbs efficiently inhibited the ELISA activity of IFN-γ, whereas an IgA mAb was ineffective. The inhibition was not influenced by a wide variety of anti-proteolytic agents but was effectively blocked by anti-complementary substances or treatments directed to the first (C1) and third (C3) component of complement. Our results indicate that activation of the classical pathway of complement (CPC) and the concomittant covalent binding of C3 to the IFN-γ molecule play a major role in the inhibitory process. It is concluded that reduction of the ELISA activity is attributable to diminished accessibility of the detector antibody for the IFN-γ protein as a consequence of C3 binding.

(KEY WORDS: ELISA, interferon-gamma, complement system)

INTRODUCTION

IFN-γ is a lymphokine produced solely by activated T lymphocytes (1) and NK-cells (2). The lymphokine has unique immunomodulatory properties and exerts its activity by

binding to a specific cell-surface receptor (3). Its actions best characterized are macrophage activation (4) and induction and/or enhancement of MHC class I and II antigen expression on a wide variety of cell types *in vitro* (5) and *in vivo* (6). IFN-γ is also an important regulatory protein for the expression of different components of the complement system including C3 (7). Recently, we have shown that incubation of rat IFN-γ in fresh normal rat serum (NRS) dramatically inhibited its ELISA activity, reducing the recovery in a sandwich ELISA system by approximately 90% (8). Heating of serum for 30 minutes at 56°C completely abrogated this inhibitory effect. The susceptibility of the ELISA system to inhibitory substances in fresh serum is a major drawback for measuring endogenously produced IFN-γ levels in serum samples, the more so as IFN-γ itself is a heat-labile protein (9, this article). The results presented in this paper show that serum-mediated inhibition can be overcome by substances or treatments which inactivate the CPC.

It is also demonstrated that serum-mediated inhibition of the ELISA activity is a consequence of covalent binding of C3 molecules to the IFN-γ protein due to CPC activation triggered by the solid-phase capture antibody. C3 binding interferes with the interaction of the detector antibody with the lymphokine reducing the sensitivity of the ELISA several fold.

MATERIALS AND METHODS

Reagents and Antisera

Ovalbumin (grade VII), bovine serum albumin (fraction V), trypsin (from bovine pancreas, type XI), trypsin inhibitor (type: 11-0), zymosan A (from S. cerevisiae), human Clq (C-0660) and human C3 (C-0651) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Polyclonal antisera directed to human complement proteins came from Dakopatts A/S, Copenhagen, Denmark. Thermolysin (art.nr. 58656) was obtained from Calbiochem, San Diego, CA, U.S.A. Cobra venom factor (CoVF) was purified from Naja naja snake venom by the method of Ballow & Cochrane (10) and

kindly provided by Dr. J. Aten, University of Leiden, The Netherlands. Production and purification of recombinant- and spleen cell-derived rat IFN-gamma (11) and the isolation and characterization of mAbs directed to rat IFN-gamma (12) and human IFN-gamma (13) have been detailed elsewhere. Preparation and specificity of rabbit antisera directed to rat complement components C1q, C3, B and H have been outlined in previous papers (14, 15). Rat C3 was purified from fresh NRS obtained from Wistar rats (16). Inactivated C3 (iC3) was obtained by overnight dialysis of C3 against PBS containing 0.5 M KSCN. The chemical was removed by further dialysis against PBS. C3b was prepared by treatment of purified C3 (500 μg/ml) with 5 μg/ml trypsin (3 min, 37°C) followed by inactivation of trypsin with 10 μg/ml soybean trypsin inhibitor.

Binding Studies

The interaction between IFN-γ and C3 was determined by ELISA methodology. Human IFN-γ or mAbs specific for IFN-γ were coated onto the wells of a microtiterplate and incubated with serum preparations or increasing amounts of purified C3. Bound C3 protein was subsequently detected with antibodies specific for the complement protein. For the rat system rat C3 was preadsorbed to the wells of a microtiterplate and incubated with increasing amounts of recombinant rat IFN-γ. Bound lymphokine was detected with a labelled monoclonal antibody specific for the rat lymphokine. The ELISA conditions were essentially as described previously (8).

RESULTS

Serum-mediated Reduction of IFN-γ ELISA Activity

Recombinant rat IFN-γ was added to freshly prepared NRS and incubated for varying lengths of time at 37°C in the wells of a microtiter plate precoated with an anti-rat IFN-γ mAb (DB-12). After incubation, the wells were extensively washed and bound rat IFN-γ was assessed with a second mAb labelled with biotin (detector antibody). The activity was compared with ELISA readings obtained from the same amount of rat IFN-γ added

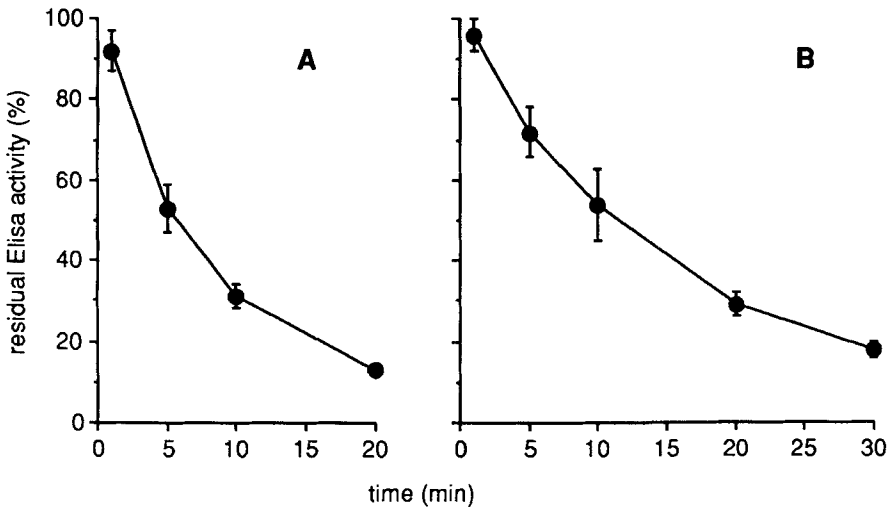

Figure 1. Kinetics of the inhibition of the ELISA activity of recombinant rat (A) and human (B) IFN-γ in fresh normal serum. The ELISA conditions for the measurement of rat (8) or human (13) IFN-γ in serum preparations have been described elsewhere.

to heat-inactivated fetal calf serum (FCS), which served as 100% controls. The time-dependent inhibition of rat IFN-γ in the ELISA system is shown in Figure 1A. Both recombinant and naturally derived rat IFN-γ were affected by the inhibitory activity to the same extent (not shown). To determine whether the observed rapid decay of the immunoreactivity is a more general phenomenon, the same experiment was performed with an ELISA system specific for human IFN-γ. Also in this case, the immunoreactivity of the human lymphokine was rapidly lost upon incubation in freshly prepared human serum (NHS; Fig. 1B). We next examined the concentration of the inhibitory substance(s) in NRS. The inhibitory activity was reduced to 50% of the original value at a serum dilution of approximately 1/64 (not shown). The inhibitor(s) turned out not to be species specific as inhibition of rat IFN-γ also occurred in fresh human serum (not shown).

Characterization of the Inhibitory Activity

In a first attempt to investigate the cause of the reduction in more detail, a number of treatments or agents were evaluated as to their capacity to eliminate the inhibitory activity. A wide variety of anti-proteolytic substances lacked activity or had only a marginal effect (not shown). By contrast a number of anti-complementary substances or treatments were highly effective. As shown in Table I, the inhibitory activity was exceedingly heat-labile. Incubation of NRS for 15 minutes at 56°C completely destroyed its activity. Mg.EGTA [10 mM ethyleneglycol-bis-(β-aminoethyl ether)N,N,N',N'-tetraacetic acid supplemented with 5 mM magnesium chloride] and monospecific polyclonal anti-rat C1q antibodies, two reagents that specifically block or prevent the activation of the CPC completely abrogated the serum-mediated inhibition (Table I). KSCN and KBr were also effective just as monospecific polyclonal antibodies directed to rat C3 or complement factor H (Table I). The inhibitory activity was absent in serum of rats, which had been pretreated with CoVF, a factor that induces complete depletion of C3 from the circulation (17). Blockade of the alternative pathway of complement (APC) by adding monospecific antibodies directed to complement factor B was not effective (Table I). It is concluded that components of the CPC play a prominent role in the inhibitory process.

CPC-activation by Plastic-adsorbed Immunoglobulins

It has recently been shown that in the absence of antigen, plate-adsorbed antibodies are able to activate the CPC in fresh serum. These experiments showed that activation is highly isotype-dependent (18). To investigate the role of the plate-coated capture antibody in the serum-mediated inhibition, three different murine Ig mAb isotypes specific for rat IFN-γ were adsorbed to the wells of a microtiter plate and incubated with fresh NRS supplemented with a predetermined amount of recombinant rat IFN-γ. These experiments are presented in Table 2 and revealed that binding of IFN-γ to an IgG_1 or IgG_{2a} mAb substantially reduced the ELISA activity of IFN-γ. On the other hand, binding to a murine IgA mAb (DB-2) had a limited effect. These results indicate that the

TABLE 1

The effects of anti-complementary substances or treatments on the ELISA activity of rat IFN-γ added to fresh normal serum

serum treatment/additions	[1]residual ELISA activity (%)
none	10 ± 2
15'56°C	99 ± 4
EDTA (10 mM)	92 ± 3
Mg.EGTA (10 mM EGTA + 5 mM Mg^{2+})	90 ± 3
zymosan	85 ± 6
KSCN (0.5 M)	98 ± 3
KBr (0.5 M)	93 ± 4
serum of a CoVF-treated rat[2]	96 ± 1
anti-rat C1q (1 mg/ml)	100 ± 3
anti-rat C3 (2 mg/ml)	95 ± 2
anti-rat factor B (2 mg/ml)	6 ± 2
anti-rat factor H (2 mg/ml)	99 ± 1
rabbit IgG (2 mg/ml)	12 ± 1

[1]8 units of rat IFN-γ was added to fresh NRS and incubated for 60 minutes at 37°C in an ELISA system specific for the rat lymphokine. The ELISA procedure has been detailed previously (8). ELISA readings obtained with rat IFN-γ added to heat-inactivated FCS and subjected to the same experimental procedure served as 100% controls.
[2]CoVF was injected i.p. 20 hours before blood was collected.

capture antibody in the ELISA system plays a crucial role in the inhibitory process and suggest that inhibition can be accomplished only with antibodies that have the intrinsic capacity to activate the CPC (have the capacity to interact with C1q).

Irreversible Inactivation of IFN-γ

To study whether complement-mediated inhibition is an irreversible process, a predetermined amount of rat IFN-γ was added to fresh NRS and heated for varying lengths of time at 56°C. This procedure was carried out before and after immobilization of IFN-γ to

TABLE 2

The effect of isotype specificity of the capture monoclonal antibody on serum-mediated inhibition of the ELISA activity of rat IFN-γ

mouse mAb isotype[1)]	mAb designation[1)]	residual ELISA activity (%)[1)]
IgG_{2a}	DB-14	8 ± 4
IgG_1	DB-12	11 ± 3
IgA	DB-2	89 ± 3

Recombinant rat IFN-γ at 8 U/ml was added to fresh NRS and incubated for 1 h at 37°C in a specific ELISA system. [1)]Both the ELISA assay (8) and the characteristics of the mAbs used as capture antibodies (12) have been detailed elsewhere. ELISA readings obtained with rat IFN-γ added to heat-inactivated FCS served as 100% controls.

the plate-adsorbed capture mAb (DB-12). Taking into account the substantial reduction of the ELISA activity of rat IFN-γ as a consequence of the elevated temperature (Fig. 2), the experiments revealed that the ELISA activity was permanently lost if the lymphokine was immobilized to the plate-adsorbed mAb (Fig. 3B). On the other hand the ELISA activity was fully recovered after 10 minutes incubation at 56°C in the absence of the DB-12 mAb (Fig. 3A), also stressing the importance of the capture antibody in the inhibitory process.

Binding Studies with Complement Proteins

So far our data indicate that activation of the CPC plays an essential role in the inhibitory process. To assess more directly the involvement of complement proteins, plate-adsorbed rat IFN-γ or rat IFN-γ immobilized to a plate-adsorbed mAb (DB-12), were incubated with fresh and heat-inactivated NRS. After extensive washing monospecific antibodies for C1q and C3 were added for the detection of bound complement proteins.

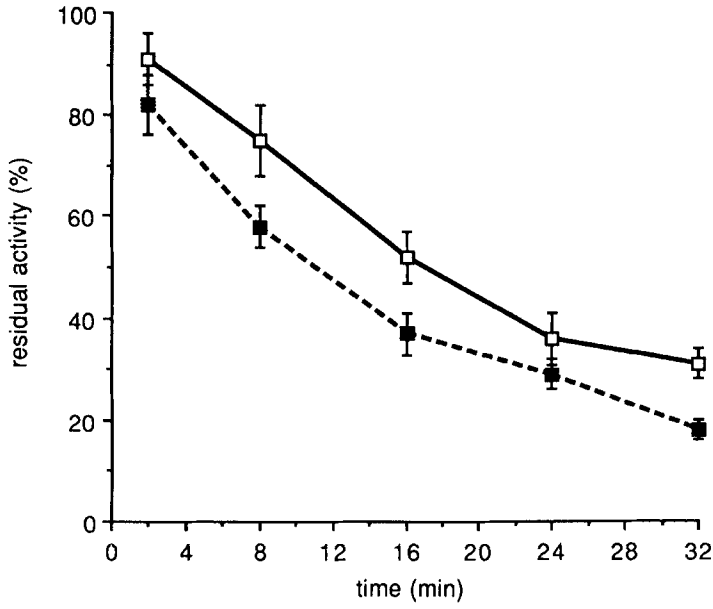

Figure 2. Effect of incubation at 56°C on the ELISA and antiviral bioactivity of rat IFN-γ. 400 Units recombinant rat IFN-γ was added to 1.0 ml heat-inactivated FCS and incubated in 10 ml flat-bottomed vials for varying length of time at 56°C. Thereafter, the residual ELISA (■) and antiviral bioactivity (□) were determined. The methods for the measurement of the antiviral bioactivity (11) and ELISA activity (8) have been detailed elsewhere.

In fresh NRS both C1q- and C3-like molecules effectively interacted with free DB-12 and with the DB-12.rat IFN-γ complex (Figs. 4 and 5). The binding of C3-like molecules to plate-adsorbed IFN-γ was detectable only with high serum concentrations (Fig. 4) whereas the binding of C1q to the lymphokine was negligible (Fig. 5). Binding of C3-like molecules to the DB-12.rat IFN-γ complex was slightly but significantly reduced as compared to free DB-12, probably as a result of diminished CPC activation due to a lowered affinity of C1q for the DB-12.rat IFN-γ complex (Fig. 5). Furthermore, there was a significant reduction of the amount of C3-like molecules that deposited onto the antibody.rat IFN-γ complex if the IgG_1 mAb (DB-12) was replaced

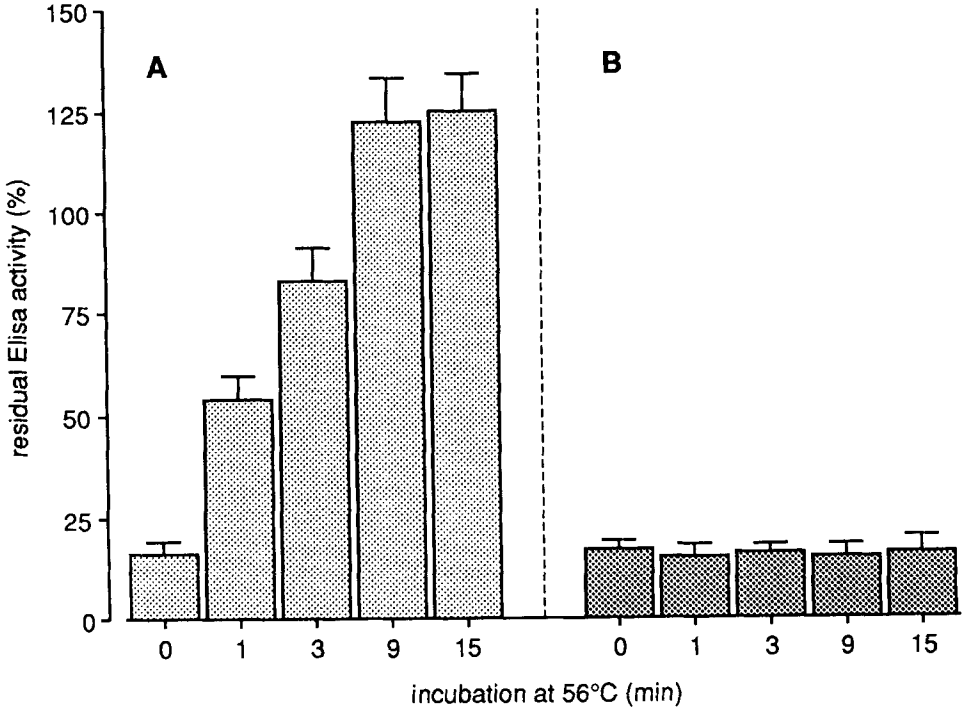

Figure 3. The effect of incubation at 56°C on serum-mediated inhibition of the ELISA activity before (A) and after (B) binding of rat IFN-γ to the capture antibody. A. Rat IFN-γ at 16 U/ml was added to fresh NRS and incubated for varying lengths of time at 56°C. Thereafter, the residual ELISA activity was measured in wells of a microtiter plate precoated with a monoclonal antibody specific for rat IFN-γ (DB-12). B. Rat IFN-γ at 16 U/ml was added to 1% ovalbumin and incubated for 1 h at 37°C in wells of a microtiter plate precoated with DB-12. After washing fresh NRS was added to the wells and the plate was heated for the indicated lengths of time at 56°C. ELISA readings of rat IFN-γ added to heat-inactivated FCS and subjected to the same experimental procedure served as 100% controls. The ELISA activity was determined as described (8).

by an IgA anti-rat IFN-γ mAb (Fig. 6). Next we examined the interaction of plate-adsorbed human IFN-γ for purified human C3. It was found that C3 bound dose-dependently to the lymphokine (not shown). Also in the rat system an interaction between plate-adsorbed C3 and purified IFN-γ could be demonstrated. The high molecular weight forms of C3 (i.e., C3b and iC3) bound rat IFN-γ to the same extent

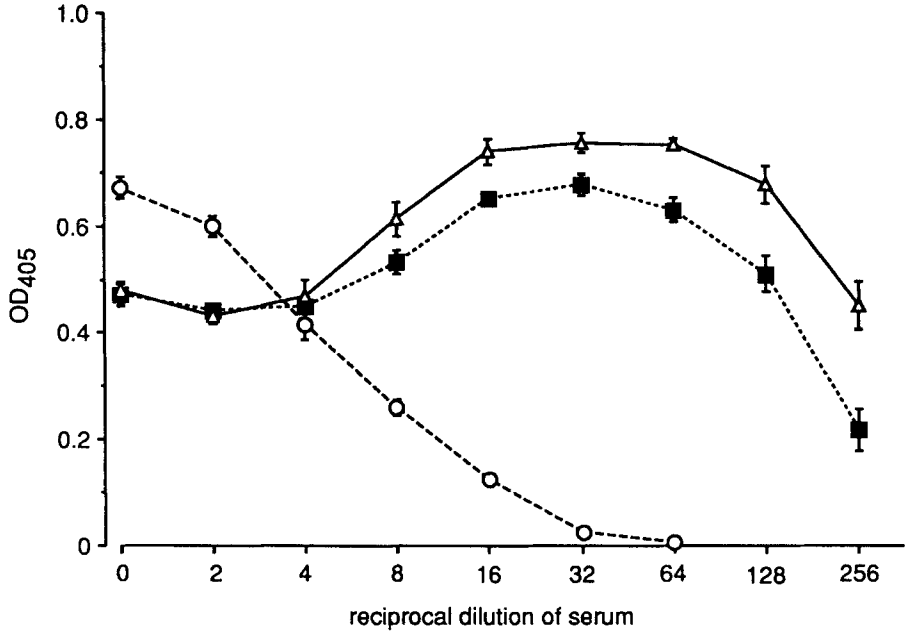

Figure 4. Binding of serum-derived C3-like molecules to plate-adsorbed rat IFN-γ (o), mAb DB-12 (Δ) or mAb DB-12 plus saturating amounts of rat IFN-γ (■) as a function of the serum concentration. Purified DB-12 or recombinant rat IFN-γ were preadsorbed to the wells of a microtiterplate at 15 μg/ml in PBS. Wells were postcoated with 2% ovalbumin. Subsequently, the half of the DB-12-coated wells were incubated with rat IFN-γ at 50,000 U/ml (100 μl/well). After washing the wells were filled and incubated with fresh or heat-inactivated NRS diluted in GVB^{2+} (veronal-buffered saline, pH 7.5 plus $5x10^{-4}$ M $magnesium^{2+}$ and 1.5×10^{-4} M $calcium^{2+}$) containing 0.5% ovalbumin. After extensive washing with PBS-Tween supplemented with 1 M NaCl, rabbit monospecific antibodies to rat C3 were added for the detection of bound C3-like molecules. ELISA readings obtained with the proper dilutions of heat-inactivated NRS are subtracted as blank values.

and in a dose-dependent way (Fig. 7). The interaction between purified C3 and IFN-γ was critically dependent on the ionic strength of the buffer. PBS supplemented with 0.5 M NaCl almost completely abrogated the binding. However, the complex between IFN-γ and C3 formed in fresh serum was not affected by a number of powerful dissociating agents (not shown). These results suggest that IFN-γ binds with a low affinity to the

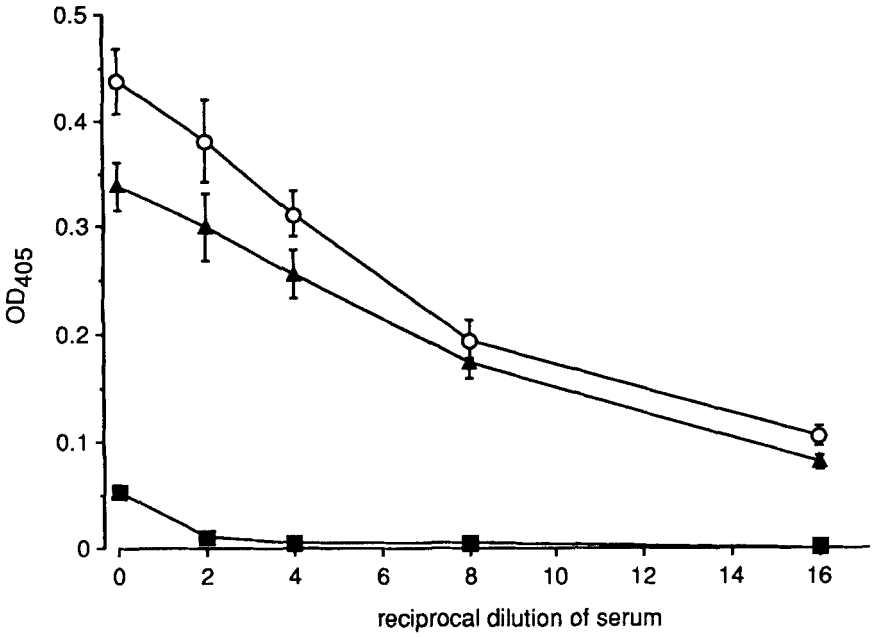

Figure 5. Binding of serum-derived C1q-like molecules to plate-adsorbed rat IFN-γ (■) mAb DB-12 (o) or mAb DB-12 saturated with rat IFN-γ (▲). Experimental conditions were essentially as described in the legend to Figure 3 on the understanding that rabbit monospecific antibodies to rat C1q were used for the detection of bound protein. ELISA readings obtained with the proper dilutions of heat-inactivated NRS are subtracted as blank values.

third component of complement and can be covalently attached to the lymphokine upon complement activation.

C3 Binding Does Not Require Biologically Active IFN-γ

To evaluate whether biologically active IFN-γ is a prerequisite for its interaction with C3, the lymphokine was digested with thermolysin, a proteolytic enzyme that removes a stretch of about 15 amino acids (estimated by SDS-PAGE) from the mature lymphokine. This procedure completely destroys the antiviral bioactivity of the lymphokine but only

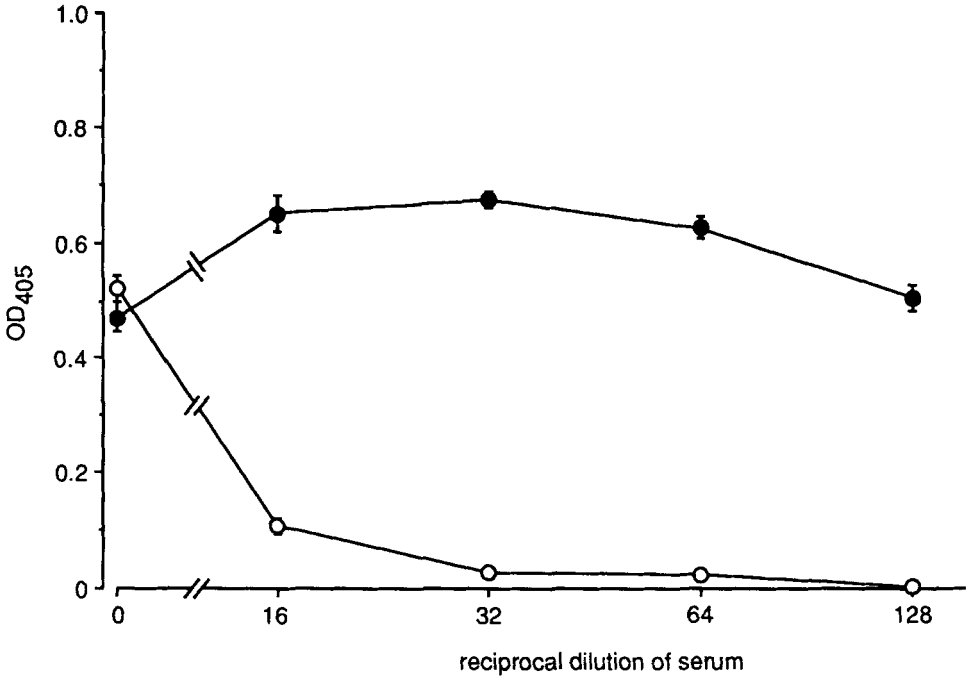

Figure 6. Binding of serum-derived C3-like molecules to plate adsorbed mAbs DB-2 (o) and DB-12 (•) both saturated with recombinant rat IFN-γ. Experimental conditions were essentially as described in the legend to Figure 3. ELISA readings obtained with the proper dilutions of heat-inactivated NRS are subtracted as blank values.

marginally affects its ELISA activity (8). After digestion the lymphokine was still susceptible to the inhibitory effects of fresh NRS in the ELISA system suggesting that the C3 binding site on the lymphokine does not interfere with the site(s) that trigger the receptor-mediated biological activity.

DISCUSSION

The complement system consists of a number of effector molecules that coordinate and mediate various immune processes in host defence and inflammation. C3 is the most

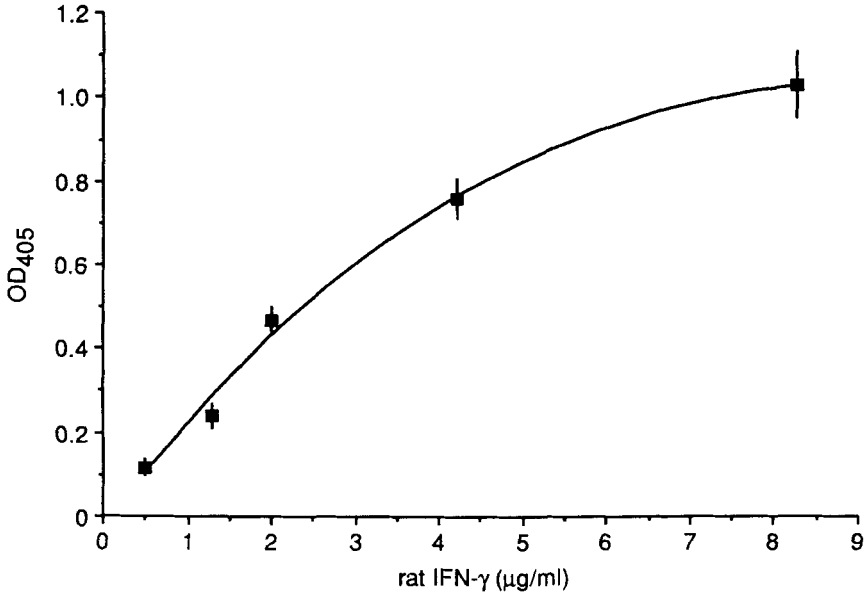

Figure 7. Binding of rec.rat IFN-γ to solid-phase immobilized rat C3b. Purified C3b was coated to the wells of a microtiter plate. Remaining binding sites were blocked with 2% ovalbumin. Varying amounts of affinity purified rat IFN-γ were added to the wells and incubated at room temperature for 60 minutes. Thereafter, bound lymphokine was detected with a labelled mAb specific for rat IFN-γ (DB-12). As controls for non-specific binding, incubations were also performed with ovalbumin-coated microwells. These ELISA readings were subtracted as blank values from the experimental values. Only the results with C3b are presented. The binding curves obtained with native C3 and iC3 were similar.

abundant complement protein in plasma and possesses a pivotal role in the activation of the system. Activation which leads to covalent binding of nascent C3b fragments onto target particles can occur by either of two pathways:

a. the classical pathway (CPC) which is activated by the binding of the first component (C1) to antigen-antibody complexes,

b. the alternative pathway (APC) which can operate without antibody participation.

The most important products in both pathways are high molecular weight fragments of C3 (e.g., C3b) as well as small split products (e.g., C3a). At least 10 different molecules

have been reported to interact non-covalently with C3 or its fragments including LPS (19), IgG (20), IL-2 (21) and a number of specific cell surface receptors (22). In the experiments described herein it is demonstrated that IFN-γ interacts dose-dependently and non-covalently with C3 and its opsonic active fragment C3b. The extent of binding was found to be identical for both molecules. A role for IFN-γ in complement activation was suggested by the observation that monoclonal antibody immobilized rat IFN-γ rapidly looses its ELISA activity upon incubation at 37°C in freshly prepared serum. This phenomenon did not stand alone as an independent ELISA system specific for human IFN-γ also revealed rapid elimination of the ELISA activity in fresh serum (Fig. 1). Serum-mediated inhibition was found to be irreversible once the lymphokine was immobilized to the plate-adsorbed capture antibody (Fig. 2).

Pretreatment of serum with agents directed to components of the CPC completely abrogated the inhibitory activity irrespective of the absence or presence of the lymphokine. Agents or treatments directly affecting the third component of complement were particularly effective in the elimination of the serum-mediated inhibition. Depletion of C3 from the circulation by administration of CoVF to normal rats produced serum without inhibitory activity. The same effect was obtained by incubation of fresh NRS with zymosan, a yeast cell wall preparation that completely consumes C3 from serum without affecting C1, C2 or C4. Also the addition of monospecific antisera against C3 or complement factor H to serum turned out to be effective procedures for the elimination of the inhibitory activity. Factor H is a regulatory protein in the APC and antibodies directed to this factor have been shown to deplete C3 hemolytic activity as a result of hypercatabolism of C3 (15). Anti-factor B blocks complement activation via the alternative pathway and did not diminish the inhibitory activity. It even tended to potentiate it (Table 1). In addition KSCN and KBr, two chemicals which convert C3 and C4 into hemolytically inactive forms (leaving all other complement proteins unaffected; 23), completely abrogated the serum-mediated inhibition (Table 1).

On the other hand agents or treatments directed to C3 were unable to affect the antiviral bioactivity of IFN-γ (not shown), indicating that the non-covalent interaction of C3 with the lymphokine has no influence on the receptor-mediated biological activity of IFN-γ. Moreover, the clearance rate *in vivo* of a predetermined amount of rat IFN-γ administered to CoVF-treated rats was identical to that in PBS-treated control rats (to be published).

It is concluded that CPC activation and the concomittant covalent binding of C3 to IFN-γ diminish accessibility of the detector antibody for the IFN-γ protein. Such a mechanism would explain the rapid decay of the immunoreactivity of rat IFN-gamma in the ELISA system while leaving the (antibody-independent) bioactivity unaffected. Although the mouse IgG_1 mAb (DB-12) used in the ELISA is not considered to be a potent activator of CPC (24), IgG_1 proteins have been found which do fix complement (25). Furthermore, replacement of the IgG_1 by a non-complement activating IgA anti-rat IFN-γ mAb almost completely abrogated the inhibition (Table 2), stressing the importance of the role of the capture antibody in the inhibitory process.

The physiological significance of the IFN-γ.C3 interaction is not clear. Binding of C3 or C3b to IgG molecules has been demonstrated (20) and it can be speculated that binding of IFN-γ to IgG.C3(b) complexes on target particles (for instance the surface of parasites) triggers CPC activation by an at present unknown mechanism and contributes to immune clearance of these pathogens (such a role of IFN-γ is under investigation). The finding that the antiviral bioactivity is not affected in fresh serum by C3 molecules and biologically inactive IFN-γ is still able to interact with C3, point to a functional property of IFN-γ that is distinct from its receptor-mediated bioactivity. Most interestingly, it has recently been demonstrated that IL-2, a lymphokine produced by the same T cell as IFN-gamma (26) also interacts with C3 (21). No significant differences in the extent of binding between IL-2 and C3 or its high-molecular weight fragment C3b could be found. In addition, normal serum reduced the sensitivity of a radioimmunoassay for human IL-2 approximately five-fold (27). It might be of interest

to determine whether this reduction in RIA activity is also based on a complement-mediated process.

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REFERENCES

1. Marcucci, F., Waller, M., Kirchner, H. and Krammer, P. Production of immune interferon by murine T cell clones from long-term cultures. Nature (Lond.) 1981; **291**: 79-82.

2. Kasahara, T., Djeu, J.Y., Dougherty, S.F. and Oppenheim, J.J. Capacity of human large granular lymphocytes (LGL) to produce multiple lymphokines: interleukin 2, interferon, and colony stimulating factor. J. Immunol. 1983; **131**: 2379-2385.

3. Anderson, P., Yip, Y.K. and Vilcek, J. Specific binding of ^{125}I-human interferon-gamma to high affinity receptors on human fibroblasts. J. Biological Chem. 1982; **257**: 11301-11304.

4. Murray, H.W., Spitalny, G.L. and Nathan, C.F. Activation of mouse peritoneal macrophages in vitro and in vivo by interferon-gamma. J. Immunol. 1985; **134**: 1619-1622.

5. Carrel, S., Schmidt-Kessen, A. and Giuffre, L. Recombinant interferon-gamma can induce the expression of HLA-DR and -DC on DR-negative melanoma cells and enhance the expression of HLA-ABC and tumor-associated antigens. Eur. J. Immunol. 1985; **15**: 118-123.

6. Steiniger, B., Falk, P. and Van der Meide, P.H. Interferon-gamma in vivo. Induction and loss of class II MHC antigens and immature myelomonocytic cells in rat organs. Eur. J. Immunol. 1988; **18**: 661-669.

7. Celada, A., Klemsz, M.J. and Maki, R.A. Interferon-γ activates multiple pathways to regulate the expression of the genes for major histocompatibility class II- 1-Aβ, tumor necrosis factor and complement component C3 in mouse macrophages. Eur. J. Immunol. 1989; **19**: 1103-1109.

8. Van der Meide, P.H., Borman, A.H., de Labie, M.C.D.C. et al. A sensitive two-site enzyme-immunoassay for the detection of rat interferon-gamma in biological fluids. J. Interferon Res. 1990; **10**: 183-191.

9. Dijkema, R., Van der Meide, P.H., Pouwels, P.H., Caspers, M., Dubbeld, M. and Schellekens, H. Cloning and expression of the chromosomal immune interferon gene of the rat. EMBO J. 1985; **4**: 761-767.'

10. Ballow, M. and Cochrane, C.G. Two anticomplementary factors in cobra venom: hemolysis of guinea pig erythrocytes by one of them. J. Immunol. 1969; **103**: 944-952.

11. Van der Meide, P.H., Dubbeld, M., Vijverberg, K., Kos, T. and Schellekens, H. The purification and characterization of rat gamma interferon by use of two monoclonal antibodies. J. gen. Virol. 1986; **67**: 1059-1071.

12. Van der Meide, P.H., Borman, A.H., Beljaars, H.G., Dubbeld, M.A., Botman, C.A.D. and Schellekens, H. Isolation and characterization of monoclonal antibodies directed to rat interferon-gamma. Lymphokine Res. 1989; **8**: 439-449.

13. Van der Meide, P.H., Dubbeld, M. and Schellekens, H. Monoclonal antibodies to human immune interferon and their use in a sensitive solid-phase ELISA. J. Immunol. Methods 1985; **79**: 293-305.

14. Veerhuis, R., Van Es, L.A. and Daha, M.R. In vivo degradation of rat C1q induced by intravenous injection of soluble IgG aggregates. Immunology 1985; **54**: 801-810.

15. Veerhuis, R., Van Es, L.A. and Daha, M.R. In vivo modulation of rat complement activities by infusion of anti-H antibodies. Immunobiol. 1985; **170**: 133-145.

16. Daha, M.R., Stuffers-Heiman, M., Kijlstra, A. and Van Es, L.A. Isolation and characterization of the third component of rat complement. Immunology 1979; **36**: 63-70.

17. Birdsey, V., Lindorfer, J. and Gewurz, H. Interaction of toxic venoms with the complement system. Immunology 1971; **21**: 299-310.

18. Zwirner, J., Felber, E., Reiter, C., Riethmüller, G. and Feucht, H.E. Deposition of complement activation products on plastic-adsorbed immunoglobulins. A simple ELISA technique for the detection of defined complement deficiencies. J. Immunol. Methods 1989; **124**: 121-129.

19. Breuscher, H.U. and Brade, V. Lipopolysaccharides as complement inhibitors by complex formation with the purified third complement component (C3). Immunobiology 1986; **173**: 41-55.

20. Kulics, J., Rajnavölgyi, E., Füst, G. and Gergely, J. Interaction of C3 and C3b with immunoglobulin G. Mol. Immunology 1983; **20**: 805-810.

21. Bartók, I., Erdei, A., Mouzaki, A. et al. Interaction between C3 and IL-2; inhibition of C3b binding to CR1 by IL-2. Immunology Letters 1989; **21**: 131-138.

22. Frank, M.M. and Fries, L.F. Complement. In: Paul, W.E., ed. Fundamental Immunology, 2nd edn. New York: Raven Press Ltd. 1989: 679-701.

23. Müller-Eberhard, H.J., Dalmasso, A.P. and Calcott, M.A. The reaction mechanism of βIC-globulin (C'3) in immune hemolysis. J. Exp. Med. 1966; **123**: 33-54.

24. Leatherbarrow, R.J. and Dwek, R.A. Binding of complement subcomponent C1q to mouse IgG_1, IgG_{2a} and IgG_{2b}: a novel C1q binding assay. Mol. Immunology 1984; **21**: 321-327.

25. Hirayama, N., Hirano, T., Kohler, G., Kurata, A., Okumura, K. and Ovary, Z. Biological activities of anti-trinitrophenyl and anti-dinitrophenyl mouse monoclonal antibodies. Proc. natn. Acad. Sci. U.S.A. 1982; **79**: 613-615.

26. Cherwinski, H.M., Schumacher, J.H., Brown, K.D. and Mosmann, T.R. Two types of mouse helper T cell clones. III. Further differences in lymphokine synthesis between T_h1 and T_h2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. J. Exp. Med. 1987; **166**: 1229-1244.

27. Cardenas, J.M., Marshall, P., Henderson, B. and Altman, A. Human interleukin 2. Quantitation by a sensitive radioimmunoassay. J. Immunol. Methods 1986; **89**: 181-189.