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ANTIBODIES TO A SYNTHETIC PEPTIDE CORRESPONDING TO THE N-TERMINAL END OF MOUSE GAMMA INTERFERON (IFNγ)

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SUMMARY: Antibodies to an N-terminal synthetic peptide of mouse γ interferon (MoIFNγ) neutralized the antiviral activity of MoIFNγ but not MoIFNα/β, human IFNα (HuIFNα), HuIFNβ or cynomologus monkey IFNγ (CynIFNγ). Comparatively, antibodies to mouse N-terminal synthetic peptide showed only 10% reciprocal cross-reactivity in neutralization tests against heterologous HuIFNγ and 13% cross-reactivity in the ELISA test against Hu N-terminal peptide. The predetermined specificity of these antibodies make them powerful tools for studying antigenic relatedness and biological properties of IFNγs.

Mouse gamma interferon (MoIFNγ) is characterized by neutralization with antibodies produced to partially purified MoIFNγ (1). These antibodies are used for classifying MoIFNγ with respect to antiviral activity, but are not fully acceptable for determining other biological properties of MoIFNγ, since the immunogen used was not fully characterized. We report the production and characterization of antibodies to a synthetic peptide whose amino acid sequence is based on the inferred 20 N-terminal amino acid sequence of MoIFNγ, determined from the cDNA structure (2). These antibodies are highly specific for MoIFNγ when compared with their reactivity to other IFNs.

Abbreviations used are:
IFN, interferon; Mo, mouse; Hu, human; Cyn, cynomolgus monkey; ELISA, enzyme linked immunoadsorbent assay.

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MATERIALS AND METHODS

Synthetic peptides. Mouse IFNγ N-terminal peptide was synthesized by Peninsula Laboratories, San Carlos, CA by the Marglin-Merrifield solid-phase method (3). The sequences of the 20 N-terminal amino acids were based on the recently identified sequence of the cDNA that codes for MoIFNγ (2).

Coupling. The synthetic peptides were coupled to keyhole limpet hemocyanin via disulfide bonds with the use of cystamine dihydrochloride (4) as previously described (5), except that derivatized MoIFNγ synthetic peptide was coupled to reduced KLH to circumvent solubility problems.

Antisera to synthetic and natural mouse and human IFNγ. Antisera to the N-terminal synthetic peptides of MoIFNγ and HuIFNγ were produced in rabbits as previously described (5). Antisera to natural mouse and human IFNγ were prepared as previously described (1,6).

Interferon assays. HuIFNs and CynIFNγ were assayed using the 50% Sindbis virus cytopathogenic effect (CPE_{50}) reduction assay previously described (7). Mouse IFNs were assayed using the 50% vesicular stomatitis virus microplaque reduction (PR_{50}) assay (8).

Interferons. Human IFNα (10^6 units/mg protein) was a gift from Dr. K. Cantell (Public Health Center, Helsinki, Finland). Crude human IFNβ ($10^{5.5}$ units/ml) and mouse IFNα/β ($10^{3.5}$ units/ml) were obtained from HEM Research, Inc. (Rockville, MD) and Litton Bionetics (Kensington, MD), respectively. Crude mouse ($10^{3.2}$ units/ml), cynomolgus monkey ($10^{2.5}$ units/ml), and human IFNγ ($10^{3.5}$ units/ml) were produced using methods previously described (7,9,10).

Neutralization of antiviral activity of IFNγ. Antibody titers against HuIFNγ, MkIFNγ and MoIFNγ were determined by mixing equal volumes of serially diluted serum with 10 CPE_{50} reduction units of HuIFNγ, MkIFNγ or with 10 PR_{50} units of MoIFNγ, incubated at room temperature for 1 hr and assayed as previously reported (1,6).

Enzyme-linked immunosorbent assay (ELISA) for antibodies specific for synthetic peptide. The ELISA used to detect antibodies to the N-terminal peptides of MoIFNγ and HuIFNγ was identical to the one previously reported for detection of antibodies to the synthetic peptide of HuIFNγ (5).

RESULTS AND CONCLUSIONS

Specificity of neutralization of various IFNs by anti-synthetic peptide serum of MoIFNγ. To compare the neutralizing specificities of antibodies made to MoIFNγ synthetic peptide, sera were assayed against various IFN preparations (Table 1). Anti-MoIFNγ synthetic peptide antiserum neutralized MoIFNγ but had no neutralizing effect (<1% of control) on 10 units of MoIFNα/β, HuIFNα, HuIFNβ, or MkIFNγ. Antibodies to synthetic peptides of MoIFNγ and HuIFNγ exhibited 9% and 8% reciprocal cross-neutralization, respectively, for their heterologous IFNγ. These results indicate that the anti-synthetic peptide antibodies were effective

Table 1

Specificity of neutralization by antisera to the synthetic peptides of MoIFNγ and HuIFNγ and to native MoIFNγ and HuIFNγ for different IFNs

	Neutralization by:			
Interferon	Anti N-terminal MoIFNγ[a] (% of control)	Anti-MoIFNγ[b] (% of control)	Anti N-terminal HuIFNγ[c] (% of control)	Anti-HuIFNγ[d] (% of control)
MoIFNγ	100[e]	100	8	1
HuIFNγ	9	0.5	100	100
MkIFNγ	<1	<1	60	50
MoIFNα/β	<1	<0.2	<0.1	<0.1
HuIFNα	<1	<0.2	<0.1	<0.1
HuIFNβ	<1	<0.2	<0.1	<0.1

[a]Anti-N-terminal MoIFNγ serum had a titer of 1,320 units/ml against MoIFNγ.
[b]Anti-MoIFNγ serum has a titer of 6,000 units/ml against MoIFNγ.
[c]Anti-N-terminal HuIFNγ serum had a titer of 10,000 units/ml against HuIFNγ.
[d]Anti-HuIFNγ serum had a titer of 20,000 units/ml against HuIFNγ.
[e]Values represent units of IFNγ neutralized by 1 ml of antiserum express in percent.

in neutralization of MoIFNγ and were highly specific for neutralization of homologous IFN.

Anti-native MoIFNγ and anti-native HuIFNγ antibodies exhibited less than 0.2 to 1% reciprocal neutralization, which suggests that antibodies to native MoIFNγ and HuIFNγ were directed toward determinants not common to both IFNs. Both anti-HuIFNγ synthetic peptide antibodies and anti-native HuIFNγ antibodies showed potent neutralization activity (50-60% of that of HuIFNγ) against MkIFNγ, suggesting a close antigenic relationship between HuIFNγ and MkIFNγ, including the N-terminus. The fact that both anti-MoIFNγ synthetic peptide and anti-native MoIFNγ antibodies did not neutralize MkIFNγ but neutralized HuIFNγ, would suggest that the shared functional site(s) of MoIFNγ and HuIFNγ are not present in MkIFNγ. The amount of cross reactivity is probably due to the 40% sequence homology in mouse and human IFNγ and N-terminal peptides (2,12). In addition, the cross neutralization observed for antisera against HuIFNγ for MkIFNγ suggest that HuIFNγ and MkIFNγ have many shared determinants, and that anti-HuIFNγ can be used in monkey model systems to study the natural functions of IFNγ.

Antigenic cross-reactivity of MoIFNγ and HuIFNγ. The antigenic cross-reactivity between MoIFNγ and HuIFNγ and the homology in amino acid sequences prompted a comparison of the various antisera in neutralization of MoIFNγ and HuIFNγ and in binding to synthetic N-terminal peptides via the ELISA test (Table 2). Three anti-MoIFNγ synthetic peptide antisera from three rabbits collected 16 weeks after the initial immunization had anti-MoIFNγ neutralization titers of 400 to 1,320 and exhibited cross-reactivities of 4 to 30% with HuIFNγ. The three anti-HuIFNγ synthetic peptide antisera had anti-HuIFNγ neutralization titers of 6,000 to 20,000 and exhibited cross-reactivities of 5 to 12% with MoIFNγ. The mean cross-reactivity of the 6 anti-peptide antisera was 10.5%. The same anti-MoIFNγ peptide antisera showed 11 to 33% cross-reactivity in the ELISA test against the synthetic peptide of HuIFNγ. The mean cross-reactivity of all 6 anti-peptide antisera was 13.5%. The results indicate that there was

Table 2

Cross reactivity of antibodies to native and N-terminal synthetic peptides of MoIFNγ and HuIFNγ in neutralization and ELISA tests

Antiserum Against	Rabbit No.	Neutralization titer against 10 units of MoIFNγ	HuIFNγ	%[a] Crossing	ELISA titer against N-terminal peptide MoIFNγ	HuIFNγ	%[b] Crossing
MoIFNγ N-terminal[c] peptide	1	400	120	30	810(0.5)[f]	270	33
	2	1320	120	9	7290(0.2)	810	11
	3	1200	60	4	2430(0.5)	270	11
HuIFNγ N-terminal[d]	1	300	6000	5	90	810(7.2)	11
	2	600	20000	3	810	21870(0.9)	4
	3	1200	10000	12	810	7290(1.4)	11
Native MoIFNγ[e]	1	6000	<10	<0.2	240(4)	<10	<4
	2	10000	30	0.3	720(7)	<10	<2
Native HuIFNγ[e]	1	180	18000	1	<10	1600 (9)	<1
	2	60	12000	0.5	<10	1200 (10)	<1

[a] % crossing determined by ratio of heterologous to homologous neutralization.
[b] % crossing determined by ratio of heterologous to homologous ELISA test.
[c] Sera collected from rabbits 14 weeks after initiation of immunization.
[d] Sera collected from rabbits 28 weeks after initiation of immunization.
[e] Anti MoIFNγ and HuIFNγ sera were collected from rabbits 16 months after initiation of immunization.
[f] Values in parentheses represent ratios of neutralization titer against homologous IFNγ to ELISA titer against corresponding synthetic peptide.

general agreement between the extent of cross-reactivity of anti-synthetic peptides in neutralization of IFNγ and in their cross-reactivity in the ELISA test against the N-terminal peptides.

Antibodies to native MoIFNγ and HuIFNγ showed one-thirtieth or less cross-neutralization and cross-reactivity in the ELISA tests than did the antibodies to the N-terminal peptides of MoIFNγ and HuIFNγ. This suggests that most of the neutralizing antibodies to the native IFNγs are directed toward additional determinants not presented by the synthetic peptides coupled to keyhole limpet hemocyanin.

The low ratio (1:1 to 1:5) in titers of anti-synthetic peptide antibodies in the neutralization and ELISA tests and the high ratio (4:1 to 10:1) of neutralization to ELISA titer with antibodies to native IFNγ may suggest that antibodies to the N-terminal end are highly specific, but do not constitute a major portion of the antibodies made to the natural IFNγ, i.e., the N-terminus of the native IFNγ may not be presented to the immune system in the same way as the coupled synthetic peptide.

Taken together, we have demonstrated that antibodies produced to synthetic peptides of INFγs can provide the qualitative specificities desirable for characterization, purification and definitive investigation of anticellular, immunoregulatory and other biological activities of IFNγ (13,14). Our findings suggest that the N-terminal end of IFNγ may be associated with the biological activity of the molecule, since monoclonal antibodies have been shown to bind to IFNγ but not inhibit its biological activity (11). As shown in these and other studies (15,16), antibodies of a predetermined specificity provides an option in studying functional sites of the IFNγ molecule that is not obtainable with monoclonal antibodies.

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