BINDING AND NEUTRALIZATION OF MONOCLONAL ANTIBODIES TO HUMAN INTERFERON ALPHA SUBTYPES.

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Abstract:

A number of antibodies raised against human interferon alpha (IFN-α) have been reported. We have characterized the binding to a panel of interferon alpha subtypes and some of their alleles by direct binding ELISA. Additionally, the binding to these subtypes in a pairwise manner has also been determined. For those antibody pairs, the correlation between the direct- and solution-binding characteristics is good. We have also determined the ability of these monoclonals to bind to IFN which is prebound to the interferon receptor chain 2 (IFNAR2). Most of the neutralizing antibodies will bind to IFNAR2 bound IFN-α, suggesting that their mode of neutralization is not through blockade of IFNAR2. When the ability of the MAbs to neutralize the various subtypes of IFN-α is examined we found that the MAbs do not neutralize subtypes to which they bind poorly. Although a particular MAb appears to bind well to several subtypes, it does not neutralize all of those subtypes. This discrepancy might be explained by kinetics of binding or due to the ability of the different subtypes to interact differentially with the receptor complex on cells.

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Materials and Methods:

Antibodies and Interferons from PBL InterferonSource inventory were used throughout the study.

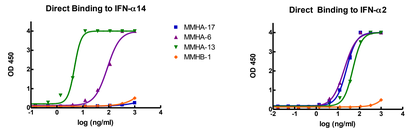
Direct binding assay. ELISA plates, Costar High Binding were coated with 1 µg/ml of each IFN subtype in PBS for 1 hour. The plates were then blocked for 1 hour with 1% BSA/PBS. Three fold dilutions of each antibody were incubated for 1 hour and then washed three times with 0.05% Tween 20/PBS. A 1/5000 dilution of Horseradish Peroxidase-Donkey anti Mouse IgG (Jackson Immunolabs) was incubated for 1 hour, washed 3 times as above and the color developed with TMB (Neogen) and the reaction was
stopped (SeyTec) after 10 minutes. Plates were read on Molecular device plate readers and data analyzed using GraphPad Prism.

IFNAR2 bridge assay. ELISA plates were coated with Donkey anti-Human Fc IgG at 5 µg/ml overnight in PBS. The plates were then blocked as above and incubated with 1 µg/ml IFNAR2-Fc (R&D Systems) for 1 hour. The plates were washed three times and then incubated with a mixture of 600 ng/ml MAb and 60 ng/ml IFN. After 1 hour the plates were washed 3 times and incubated with HRP-Anti Mouse IgG for 1 hour followed by 3 washes. Color was developed and data analyzed as above.

Sandwich assay. ELISA plates were coated with 1-2 µg/ml MAb in various buffers. Plates were blocked as above and then incubated with various IFN subtypes. After 3 washes 1 µg/ml of a Biotinylated MAb was added and incubated for 1 hour. After 3 washes, streptavidin HRP was added and incubated for 1 hour.
After 3 washes the plates were developed and analyzed as above.

iLite™ Assay. This assay is a cell based reporter gene assay using the ISG15 promoter upstream of luciferase. The assay was performed according to the manufacturers protocol. Briefly, cells were incubated with 100 U/ml of each IFN and 10 µg/ml of each MAb for 17 hours. The cells were lysed and luciferase activity determined. Cells incubated with 100 U/ml IFN were taken as 0% neutralization. Cells incubated without IFN were taken as 100% neutralization. The antibody was scored as neutralizing for a particular subtype when greater than 50% neutralization was achieved.

A549/EMCV Assay. A549 cells were plated at 2e4 cells/well. IFN subtypes at 100 or 10 U/ml were preincubated with 50 µg/ml MAb for 1 hour and then added to the cells. After 16 hours EMCV was added And after 48 hours the remaining live cells were stained with crystal violet. Protection from the virus was determined microscopically. The antibody was scored as neutralizing for a particular subtype when greater than 50% neutralization was achieved.



Monoclonal antibodies raised against human IFN-α differ in their binding to the different IFN-α subtypes. MMHA-13 binds better to IFN-α14 than to IFN-α2, and MMHA-6 bind well to both. MMHA-17 does not bind well to IFN-α14.

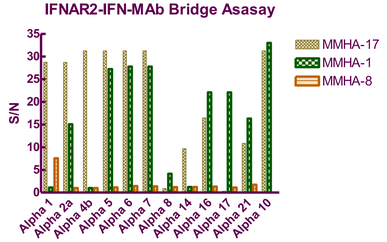
Direct Binding Results

	HA-11	HA-2	HA-14	HA-1	HA-3	HA-6	HA-13	HA-17
A2a EC50 ng/ml	37.8	19.5	5.6	52.7	12.1	20.0	35.0	21
A1b	0.00	0.3	2.4	0.2	ND	ND	ND	ND
A2a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
A2b	2.4	0.7	1.7	0.4	1.4	0.9	1.4	0.2
A4a	7.9	4.3	2.4	0.5	0.00	0.1	5.4	1.2
A4b	0.00	1.7	1.6	0.1	0.00	0.02	1.0	0.5
A5	3.6	3.9	2.8	1.6	1.0	0.9	2.3	1.0
A6	3.3	1.0	0.9	3.2	0.01	0.2	3.9	0.3
A7	3.3	1.1	3.0	0.2	0.3	0.1	0.5	0.3
A8	1.6	0.00	0.8	8.4	0.00	0.04	0.6	0.00
A10	1.5	4.2	4.4	6.2	0.01	0.1	0.1	0.9
A14	12.6	0.4	0.0	0.0	1.1	0.2	10.3	0.00
A16	2.1	0.6	2.5	0.5	1.1	0.2	0.2	0.01
A21	0.01	1.3	1.3	0.8	1.2	0.2	0.8	0.4
A17	0.5	ND	0.3	1.1	0.1	0.2	3.2	3.1

Direct binding of MAbs was assessed relative to binding to IFN-α2a. The EC_{50} for IFN-α2a was divided by the EC_{50} for the other IFNs to obtain a relative binding pattern. For all subtypes, at least one antibody bound well.

Color code: red <0.1; pink, 0.1-0.4; pale yellow, 0.5 to 1.9; dark yellow, 2-3.9; pale green, 4-9.9; dark green >10.

IFNAR2-IFN-MAb Bridge Asasay

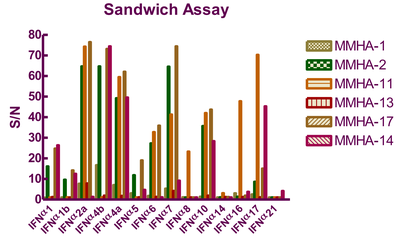


These three monoclonal antibodies bind to an IFNAR2-IFN complex. MMHA-8 is relatively selective for A1. Antibodies were tested at 600 ng/ml MAb precomplexed with 60 ng/ml IFN. The S/N is the ratio of the OD450 for complex containing IFN divided by a background determined in the absence of IFN.

Summary Results from the IFNAR2 Bridge Assay. MMHA-3 and MMHA-6 may bind to IFN at or near the IFNAR2 binding site and hence do not form a bridge. Highlighted in red are anomalous results. MMHA-11 binds well to A2a and A5 in the direct binding assay while it does not bind well to A21. Likewise MMHA-17 does not bind well to A14 in the direct binding assay but it does in this assay.

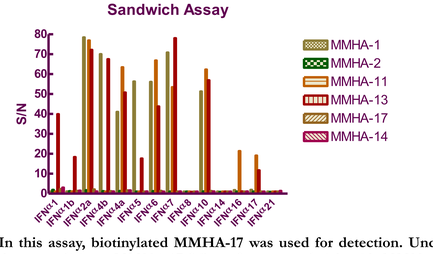
	HA-11	HA-2	HA-14	HA-1	HA-3	HA-6	HA-13	HA-17	HA-8
Alpha 1	1.14	34.66	3.34	1.12	0.96	1.16	12.84	28.75	7.56
Alpha 2a	24.84	23.55	7.00	15.06	1.30	1.25	11.57	28.80	1.00
Alpha 4b	24.84	38.46	18.41	1.01	1.24	1.17	20.20	31.25	1.01
Alpha 5	1.58	38.46	15.39	27.24	1.09	1.04	20.20	31.25	1.14
Alpha 6	24.84	38.46	6.68	27.78	1.11	1.16	20.20	31.25	1.44
Alpha 7	24.84	38.46	18.75	27.78	1.17	1.38	20.20	31.25	1.39
Alpha 8	12.26	1.20	6.54	4.17	1.06	1.08	10.04	0.90	1.21
Alpha 10	24.84	35.80	33.00	19.10	1.20	1.00	25.10	31.25	ND
Alpha 14	9.82	18.32	0.91	1.24	1.01	1.17	12.05	9.70	1.25
Alpha 16	12.45	30.21	14.80	22.10	1.32	1.36	12.78	16.46	1.34
Alpha 17	14.23	32.14	14.82	22.10	0.88	1.21	11.65	0.32	1.12
Alpha 21	8.06	22.88	5.41	16.33	1.46	1.09	13.25	10.86	1.74
Neutralizing	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes
IFNAR2 Block	No	No	No	No	Yes	Yes	No	No	No

Sandwich Assay



In this assay, biotinylated MMHA-13 was used to detect IFN bound to various monoclonal antibodies pre-coated on plates. MMHA-13 is able to form a sandwich with each of these partners (with the exception of MMHA-13 itself).

Sandwich Assay



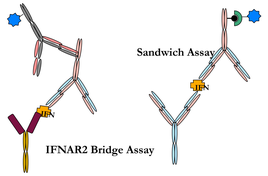
In this assay, biotinylated MMHA-17 was used for detection. Under these conditions, MMHA-17 fails to form a sandwich with MMHA-2, MMHA-14 or itself.

	MMHA-11	MMHA-11	MMHA-11	MMHA-11	MMHA-11
	Direct Binding	IFNAR2 Bridge	Any Sandwich	iLite	NAb
A1a	ND	ND	NO	Yes	positive
A1b	0.0	1.1	NO	NO	negative
2a	1.0	24.8	YES	ND	positive
2b	2.4	ND	0.0	Yes	positive
A4a	7.9	24.8	NO	Yes	positive
A4b	0.0	ND	YES	NO	negative
A5	3.6	1.6	NO	NO	negative
A6	3.3	24.8	YES	Yes	positive
A7	3.3	24.8	YES	Yes	positive
A8	1.6	12.3	YES	Yes	positive
A10	1.5	24.8	YES	Yes	positive
A14	12.6	9.8	YES	Yes	negative
A16	2.1	12.4	YES	NO	pos/neg
A17	0.5	14.2	YES	NO	positive
A21	0.0	8.1	NO	NO	pos/neg

MMHA-11 binds to most subtypes and at least weakly neutralizes most.

Subtype	MMHA-1 Direct	MMHA-1 IFNAR2	MMHA-1 Any Sandwich	MMHA-1 iLite	MMHA-1 Nab2
A1a	ND	ND	NO	ND	positive
A1b	0.2	1.1	NO	NO	positive
2a	1.0	15.1	YES	Yes	positive
2b	0.4	ND	ND	Yes	positive
A4a	0.5	1.0	YES	Weak	positive
A4b	0.1	ND	YES	Yes	positive
A5	1.6	27.2	YES	Yes	positive
A6	3.2	27.8	YES	Yes	positive
A7	0.2	27.8	YES	Weak	positive
A8	8.4	4.2	NO	No	negative
A10	6.2	19.1	YES	Yes	positive
A14	0.0	1.2	NO	No	negative
A16	0.5	22.1	YES	Yes	positive
A17	0.0	22.1	YES	Yes	positive
A21	0.8	16.3	NO	NO	positive

MMHA-1 binds to most subtypes except IFN-α14 . It binds well to IFN-α8 but does not neutralize IFN-α8 .



Subtype	MMHA-14 Direct Binding	MMHA-14 IFNAR2 Bridge	MMHA-14 Any Sandwich	MMHA-14 iLite	MMHA-14 NAb
A1a	ND	ND	YES	Yes	positive
A1b	2.4	3.3	YES	NO	positive
2a	1.0	7.0	NO	NO	positive
2b	1.7	ND	0.0	NO	positive
A4a	2.4	18.4	YES	Yes	positive
A4b	1.6	0.0	YES	YEs	positive
A5	2.8	15.4	YES	Yes	positive
A6	0.9	6.7	NO	Weak	positive
A7	3.0	18.7	YES	NO	pos/neg
A8	0.8	6.5	NO	NO	negative
A10	4.4	33.0	YES	Yes	positive
A14	0.0	0.9	NO	Weak	negative
A16	2.5	14.8	YES	NO	negative
A17	0.0	14.8	YES	NO	positive
A21	1.3	5.4	YES	NO	positive

MMHA-14 binds relatively well to many subtypes. It does not appear to neutralize IFN-α8 or IFN-α16

Conclusions

Binding to the IFN-Alpha subtypes was determined by 3 methods.
- Direct binding to IFN coated plates
- Binding to IFNAR2 coated plates bridged by the IFN subtypes
- Sandwich assay using MAb coated plates.

In general the binding results match well. Some direct binding results may be altered by conformational changes of the IFN on plate coating.

Neutralization was determined by 2 methods.
- A reporter gene assay based on ISG-15.
- Neutralization of CPE in the A549/EMCV system

The neutralization results are similar.

However, in at least two cases we have identified a disconnect between binding and neutralization. Two antibodies which bind very well to IFN-α8 do not appear to block the activity. One of these antibodies binds well to IFN-α16 and also does not neutralize the activity.

There have been suggestions in the literature that IFN-α8 may interact with the receptor differently than IFN-α2. Binding without neutralization for a neutralizing antibody lends further support to this notion.