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Neutralizing Type I IFN Antibodies Trigger an IFN-Like Response in Endothelial Cells

Herwig Peter Moll, Harald Freudenthaler, Anna Zommer, Elisabeth Buchberger, and Christine Brostjan

Neutralizing Abs to type I IFNs are of therapeutic significance, i.e., are currently evaluated for the treatment of autoimmune diseases with pathogenic IFN-α production such as for systemic lupus erythematosus. Unexpectedly, we observed that several neutralizing Abs reportedly known to counteract IFN-α or IFN-β activity triggered an “IFN-like” response in quiescent primary human endothelial cells leading to activation of the transcription factor IFN-stimulated gene factor 3 and the expression of IFN-responsive genes. Furthermore, these Abs were found to enhance rather than inhibit type I IFN signals, and the effect was also detectable for distinct other cell types such as PBMCs. The stimulatory capacity of anti-IFN-α/β Abs was mediated by the constitutive autocrine production of “subthreshold” IFN levels, involved the type I IFNR and was dependent on the Fc Ab domain, as Fab or F(ab’)2 fragments potently inhibited IFN activity. We thus propose that a combined effect of IFN recognition by the Ab paratope and the concomitant engagement of the Fc domain may trigger an IFN signal via the respective type I IFNR, which accounts for the observed IFN-like response to the neutralizing Abs. With respect to clinical applications, the finding may be of importance for the design of recombinant Abs vs Fab or F(ab’)2 fragments to efficiently counteract IFN activity without undesirable activating effects. The Journal of Immunology, 2008, 180: 5250–5256.

The inducible IFN response and the associated antivirus, antitumor, and immunomodulatory activities are well-characterized hallmarks of the defense system. These activities are primarily mediated by the rapid activation of the transcription factor IFN-stimulated gene factor (ISGF)3, which binds to promoter IFN-stimulated response elements (ISREs) and induces expression of IFN response genes such as IFIT-1 (IFN-induced protein with tetratricopeptide repeats 1) or IFN-stimulated gene (ISG)15. In contrast, the low level, constitutive expression of type I IFNs (IFN-α or IFN-β) has now been recognized to serve distinct functions in cellular signaling and activation (1): in the absence of any known stimulus, a low basal expression level of type I IFNs is maintained, which results in a weak signaling event and intracellular tyrosine phosphorylation of the type I IFN receptor α-chain (IFNAR-1). The signal is considered to be “subthresh-

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4 Abbreviations used in this paper: ISGF, IFN-stimulated gene factor; EC, endothelial cell; HDMEC, human dermal microvessel EC; IFIT-1, IFN-induced protein with tetratricopeptide repeats 1; IFNAR, IFN-α receptor; IFNGR, IFN-γ receptor; ISG, IFN-stimulated gene; ISRE, IFN-stimulated response element; siRNA, small interfering RNA.

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Technologies) and cultured in fibronectin-containing Microvascular Endothelial Cell Growth Medium EGM2-MV (Cambrex) without vascular endothelial growth factor supplementation. Purified EC cultures showed ≥98% purity and viability. For separation of lymphatic and blood vessel ECs, anti-podoplanin Ab-coupled Dynabeads were applied. All isolates were characterized by flow cytometry for EC characteristics, i.e., CD31 expression, CD34 as marker of microvessels and for E-selectin expression to keep the passage number low, experiments were generally repeated with different EC isolates, i.e., represent biological replicates with variable inducibility.

HUVECs were obtained from Cambrex and grown in fibronectin-containing EGM2 medium without vascular endothelial growth factor supplementation (Cambrex). 293T cells are derived from human embryonic kidney cells into which the temperature sensitive gene for simian virus SV40 tumor Ag was inserted. HT-29 was isolated from a human colorectal adenocarcinoma. Both cell lines (293T, HT-29) were supplied with American Type Culture Collection and cultured in DMEM with 10% FCS. PBMCs were isolated from 100 ml EDTA-treated whole blood of a healthy volunteer by standardized density gradient centrifugation using Ficoll-Paque (GE Healthcare) and were supplied with RPMI 1640 medium containing 10% FCS.

Treatment with neutralizing Abs and rIFN

Two days before stimulation, ECs were seeded in growth medium at 7 × 10^6 cells per 30-mm dish (293T and HT-29 cells at 2 × 10^6 cells) to yield a confluent cell layer within 24 h. Culture medium was then exchanged to EGM2-MV containing 5% FCS but no additional growth factor supplements and cells were allowed to adopt a quiescent state over the next 24 h. Abs were added on day 3 at various concentrations and for the time periods indicated. A total of 7 × 10^3 PBMCs in 500 μl of RPMI 1640 medium were stimulated immediately after isolation without prior culture period.

The rIFN and blocking mAbs targeting either IFN-α (clones 2 and 13, MMHA-2 and MMHA-13, respectively) or IFN-β (clones 3 and 12, MMHA-3 and MMHA-12, respectively) encoded as the IFN-α receptor (IFNAR) (MMHAR-2) were all obtained from PBL Biomedical Laboratories. ELISA kits for detection of human IFN-α or IFN-β were also manufactured by PBL Biomedical Laboratories and assays were conducted essentially as described (10). Neutralizing Abs to IFN-γ (NIB42) or the IFN-γ receptor (IFNGR) α-chain (GIR-208) were supplied by BD Biosciences. For mouse IgG1 isotype control, the MOPC-21 clone was applied (Sigma-Aldrich). TNF-α was provided by H. R. Alexander (National Cancer Institute, Bethesda, MD), whereas LPS was obtained from Invitrogen Life Technologies.

Results

Primary ECs as isolated from human dermal microvessels (HDMECs) were subjected to standard in vitro culture. When cells were exposed to increasing doses of neutralizing Abs directed against IFN-α or IFN-β (in the absence of any exogenously added type I IFN) an “IFN-like” response was observed (Fig. 1): the dose-dependent induction of IFN-responsive genes such as IFIT-1 and ISG15 was detected at the mRNA level by real-time RT-PCR as well as at the protein level by immunoblotting. The effect was verified for four different, commercially available blocking mAbs targeting either IFN-α (clone nos. 2 and 13) or IFN-β (clone nos. 3 and 12) as illustrated in Fig. 1 (also see Fig. 4). Furthermore, the stimulatory capacity of anti-IFN-α and anti-IFN-β mAbs was additive (Fig. 2A) and IFIT-1 mRNA induction was observed at all time points (2, 4, 6, and 8 h) investigated (data not shown). All
mAbs tested were of the mouse IgG1 isotype with κ light chain. An appropriate isotype control did not induce IFIT-1 or ISG15 expression in HDMECs.

A first indication on the potential mechanism underlying the extraordinary "intrinsic" ability of the type I IFN Abs to induce IFN-responsive genes came from the observation that IFIT-1 induction by Ab treatment was statistically significant ($p \leq 0.03$) for all mAbs and concentrations. Comparably, $p \leq 0.01$ was recorded for ISG15 induction with the exception of anti-IFN-α mAb clone no. 13 at the lowest concentration ($p$ was not significant). Exposure of ECs to the IFN-neutralizing Abs was well tolerated and did not result in any apparent changes in morphology (Fig. 2D) or signs of apoptosis (data not shown) over prolonged time periods. Concomitant proinflammatory activation of ECs by LPS or TNF-α led to a partial reduction but could not prevent the endothelial IFN-like response to the Abs (Fig. 3A).

The tested cell isolates mostly consisted of a mixture of vascular and lymphatic ECs originating from human skin microvessels. When analyzed in separate cultures, comparable responses were elicited in both cell populations (Fig. 3B). In addition, endothelial cultures derived from larger vessels (HUVECs) were highly responsive to the type I IFN Abs. When investigating other human cell types, we found that the phenomenon was not restricted to ECs but could also be observed in freshly isolated PBMCs when treated with the respective IFN blocking mAbs (Fig. 3C). In contrast, the

**FIGURE 1.** Dose-dependent induction of IFN-responsive genes by neutralizing Abs to type I IFN. EC cultures were treated for 4 h with 0.8, 3, or $12 \mu$g/ml anti-IFN-α mAb clone no. 2 ($n = 7$ comparable experiments) (A), clone no. 13 ($n = 2$ comparable experiments) (B), or anti-IFN-β mAb clone no. 3 ($n = 3$ comparable experiments) (C). The nonspecific mouse IgG1 isotype control was applied at $12 \mu$g/ml. Real-time RT-PCR analysis of endothelial RNA was performed to evaluate mRNA levels of IFIT-1 and ISG15. Each sample was assayed in triplicate, and comparable experiments were performed. IFIT-1 induction by Ab treatment was statistically significant ($p \leq 0.03$) for all mAbs and concentrations. Comparably, $p \leq 0.01$ was recorded for ISG15 induction with the exception of anti-IFN-α mAb clone no. 13 at the lowest concentration ($p$ was not significant). An asterisk indicates nonspecific, cross-reactive protein.

**FIGURE 2.** Characterization of the mechanisms involved in the induction of IFN response genes by neutralizing Abs to type I IFN. A, HDMECs were challenged for 4 h by single or combined treatment with $3 \mu$g/ml anti-IFN-α mAb clone no. 2 and anti-IFN-β mAb clone no. 3 (or mouse IgG1 isotype control) ($n = 3$ comparable experiments). Endothelial RNA and corresponding cDNA were then analyzed for IFIT-1 mRNA expression by real-time PCR, which demonstrated an additive effect of anti-IFN-α and anti-IFN-β Abs. B, IFIT-1 induction by $3 \mu$g/ml anti-IFN-α mAb clone no. 2 was blocked by the addition of anti-IFNAR Ab at $1 \mu$g/ml vs mouse IgG2a isotype control ($n = 3$ comparable experiments). C, Activation of the IFN responsive transcription factor ISGF3 was evaluated by reporter gene assay measuring luciferase activity in relative light units (RLU) after EC stimulation for 4 or 24 h with $6 \mu$g/ml anti-IFN-α mAb clone no. 2 or $100 \mu$g/ml rIFN-α for comparison ($n = 2$ comparable experiments). D, Endothelial cultures after 0, 4, or 24 h of stimulation with $6 \mu$g/ml anti-IFN-α mAb clone no. 2 were investigated by phase contrast microscopy to document that no apparent changes in EC morphology were triggered by Ab treatment.
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locus. Having established that endogenously expressed type I IFN me-

FIGURE 3. IFN-like response to neutralizing Abs by different EC variants or other cell types. A, HDMECs stimulated with proinflammatory mediators such as LPS at 1 µg/ml or TNF-α at 100 ng/ml were concomitantly treated with 12 µg/ml anti-IFN-α mAb clone no. 2 for 4 h (n = 2 comparable experiments). B, EC populations isolated from blood (BECs) or lymphatic (LECs) skin vessels or HUVECs were exposed to 12 µg/ml anti-IFN-α mAb clone no. 2 or mouse IgG1 isotype control for 4 h (n = 2 comparable experiments). Comparably, freshly isolated PBMCs or in vitro cultures of 293T and HT-29 cells were exposed to 12 µg/ml anti-IFN-α mAb clone no. 2 for 4 h (n = 2 comparable experiments). Total RNA and corresponding cDNA were analyzed for IFIT-1 mRNA expression by real-time PCR.

effect was essentially absent in Ab-treated 293T or HT-29 cell cultures representing a human embryonic kidney and colon carcinoma cell line, respectively.

Because all the Abs tested on HDMECs were established neutral-

izing monoclonals, we proceeded to test their blocking abilities in EC combination treatment with rIFN and mAb (Fig. 4). Four hours of incubation with rIFN-β (10 pg/ml) induced IFIT-1 mRNA levels by ~40-fold. Interestingly, addition of blocking mAbs (at 12 µg/ml) targeting IFN-β resulted in a further increase of IFIT-1 expression by 2- to 10-fold depending on the Ab applied (clone nos. 12 and 3, respectively). A similar phenomenon was observed when combining rIFN-α with anti-IFN-α blocking mAbs. In con-

contrast, a neutralizing Ab directed against the type I IFNR, IFNAR,

completely abrogated the endothelial response to rIFN-α. Further-

more, when IFIT-1 expression was triggered by type II rIFN (10,000 U/ml, rIFN-γ), the induction was efficiently blocked by the addition of neutralizing Ab targeting either IFN-γ or the corresponding type II receptor.

As the ability of the type I IFN Abs to induce IFN-responsive genes was not dependent on but could be enhanced by exog-

enously added IFN, we hypothesized that ECs might constitutively express low levels of autocrine type I IFN contributing to the effects observed. To test our hypothesis, silencing of IFN-β gene expression was achieved by transient transfecting HDMEC cul-

tures with double-stranded siRNAs. When HDMECs were chal-

lenged with IFN-β siRNA or nonspecific (control) siRNA, no in-

duction of IFIT-1 was observed, i.e., there was no endothelial response to the uptake of chemically modified dsRNA oligonucle-

otides (Fig. 5A). At 24 h posttransfection, ECs were exposed to anti-IFN-α or anti-IFN-β mAbs at 12 µg/ml. Induction of IFIT-1 mRNA was markedly inhibited by IFN-β gene silencing as opposed to control siRNA treatment (Fig. 5A): IFITT-1 expression levels in response to type I IFN Abs were reduced to ~40%. The IFN silencing efficiency of these samples was evaluated by real-

time RT-PCR analysis and equaled the effect seen for IFIT-1. However, not only IFN-β mRNA levels but also IFN-α1 and IFN-α2 transcripts were decreased by ~60% in the presence of IFN-β siRNA (Fig. 5D). In contrast, the detectable mRNA expres-
sion of nonrelated EC genes, such as the transcriptional regulator BCoR or the housekeeping gene β-actin, was not affected by IFN-β vs control siRNA (Fig. 5E).

FIGURE 4. Enhanced response to rIFN-α or rIFN-β in the presence of neutralizing Abs to type I IFN. A, HDMECs were stimulated with rIFN-β (10 pg/ml) for 4 h in the absence or presence of anti-IFN-β mAb clone no. 3 or clone no. 12, or mouse IgG1 isotype control at 12 µg/ml each (n = 3 comparable experiments). B, EC treatment for 6 h with rIFN-α (10 pg/ml) was combined with 12 µg/ml mouse IgG1 isotype control, anti-IFN-α mAb clone no. 2 or anti-IFNAR Ab (n = 2 comparable experiments). C, Comparably, ECs were exposed for 6 h to 10,000 U/ml rIFN-γ and 12 µg/ml mouse IgG1 isotype control, anti-IFN-γ, or anti-INFGR Ab (n = 3 comparable experiments). Endothelial RNA was analyzed for IFIT-1 mRNA expression by real-time RT-PCR.
fact that all our in vitro experiments were conducted in the presence of 5% FCS in culture medium thus supplying an excess of bovine IgG, binding of the mouse monoclonal anti-IFN Abs to human endothelial FcγRs could not be excluded. We therefore added increasing concentrations of mouse IgG1 isotype Ab to our reactions (Fig. 6B). A dose-dependent decline in EC responsiveness, i.e., in IFIT-1 mRNA induction by IFN-neutralizing mAbs, was observed. This response prompted us to further investigate the requirement for the Fc domain, thus we generated Fab as well as F(ab\(^\prime\))\(_2\) fragments from anti-IFN-α blocking mAb clone no. 2 by ficin digest. When comparing the Fab and F(ab\(^\prime\))\(_2\) fragments to the intact Ab (subjected to a mock treatment without addition of ficin protease for control) the intact molecule retained its dose-dependent stimulatory capacity, whereas the corresponding Fab or F(ab\(^\prime\))\(_2\) fragments could not induce IFIT-1 mRNA expression in HDMECs (Fig. 6A). However, the generated fragments exhibited strong neutralizing capacity for rIFN-α, which was not observed when Fab or F(ab\(^\prime\))\(_2\) fragments of the control mouse IgG1 isotype Ab were applied (Fig. 6C). IFIT-1 mRNA induction in response to EC treatment with 10 pg/ml rIFN-α was entirely abolished by 1 μg/ml Fab or F(ab\(^\prime\))\(_2\) fragments from anti-IFN-α blocking mAb clone no. 2, which documents the actual IFN-neutralizing potency attributed to the original Ab by the manufacturer. In comparison, the fragments were less potent in competing, or inhibiting, the activity of the intact Ig molecule. Increasing the amount of Fab fragment while maintaining the concentration of intact anti-IFN-α mAb clone no. 2 at 1 μg/ml led to a 64% drop in IFIT-1 transcript levels at a ratio of 2:1. In contrast, a comparable amount of unrelated control Fab fragment led to a nonspecific quenching of IFIT-1 induction in the range of 25% (Fig. 6B).

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** IFN-β gene silencing inhibits the IFN-like response of ECs to IFN-neutralizing mAbs. A. Following EC transfection with IFN-β siRNA or nonspecific control siRNA, cells were challenged with 12 μg/ml anti-IFN-α mAb clone no. 2 or anti-IFN-β mAb clone no. 3 for 2 h (n = 3 comparable experiments). B. To reverse the effect of IFN-β gene silencing, EC cultures were pretreated with subthreshold concentrations of rIFN-β (1 pg/ml) for 2 h with or without subsequent addition of 12 μg/ml IFN-β mAb clone no. 3 for another 2 h (n = 2). C. Treatment of siRNA transfected ECs with high-dose (100 pg/ml) rIFN-α or rIFN-β for 4 h (n = 4 comparable experiments) was followed by RNA isolation and real-time RT-PCR detection of IFIT-1 expression. D. Silencing efficiencies for IFN-β as well as IFN-α1 and IFN-α2 were determined by real-time RT-PCR and are shown for EC samples stimulated for 2 h with 12 μg/ml anti-IFN-β mAb clone no. 3 (n = 3). E. Comparably, the effect of IFN-β siRNA vs nonspecific control siRNA was tested on transcript levels of nonrelated EC genes such as BCoR and β-actin for the same samples (n = 3 comparable experiments).

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Ab domains involved in IFIT-1 induction by IFN-neutralizing Abs. A, HDMECs were exposed for 4 h to 1, 3, or 6 μg/ml of intact (control-treated) anti-IFN-α mAb clone no. 2 or a generated Fab or F(ab\(^\prime\))\(_2\) fragment of clone no. 2. Incubation for 4 h was followed by real-time RT-PCR analysis of endothelial RNA for IFIT-1 mRNA expression (n = 4 comparable experiments). In comparison to the full-length Ab, the fragments were incapable of eliciting IFIT-1 mRNA expression. p ≤ 0.029 for all concentrations of intact mAb vs fragments applied. B, The full-length anti-IFN-α mAb clone no. 2 (1 μg/ml) was mixed at a ratio of 1:1 or 1:2 with the corresponding generated Fab fragment, a nonspecific pool of unrelated Fab fragments or with an intact mouse IgG1 isotype control (n = 4 comparable experiments). C, HDMEC stimulation with rIFN-α at 10 pg/ml for 4 h was challenged with concomitant administration of 1 μg/ml Fab or F(ab\(^\prime\))\(_2\) fragment generated from anti-IFN-α mAb clone no. 2 or from a mouse IgG1 isotype Ab (n = 3 comparable experiments).
Discussion

In the study presented, we have investigated the unexpected induction of IFN-regulated genes IFIT-1 and ISG15 in primary ECs that were exposed to Abs known to neutralize type I IFN. Despite the absence of exogenously added IFN, an “IFN-like” signal was observed involving the type I IFN receptor and leading to the activation of the transcription factor ISGF3. The potency of eliciting this response varied among the Abs applied, apparently relating to differences in binding affinities rather than the specificity for IFN-α or IFN-β. Depending on the clone, Ab concentrations in the range from 1 to 10 μg/ml were sufficient to induce IFIT-1 mRNA levels comparably achievable by EC stimulation with 10 pg/ml rIFN-β (40-fold). It is of interest to note that plasma levels of neutralizing Abs reached in clinical applications are well within or even higher than the concentration range tested in our in vitro setting (16, 17).

Because all Abs applied in our analyses were reported to neutralize IFN bioactivity, as confirmed by the manufacturer in cytotoxic effect inhibition assays and as subsequently verified for the Fab and F(ab’)2 fragments in our experiments, we investigated the combined application of rIFN and mAb on HDMECs. The full-length type I IFN blocking Abs were found to enhance rather than inhibit the endothelial response to IFN-α or IFN-β. In contrast, neutralizing Abs targeting IFN-γ or the IFNRs IFNAR and IFNGR potently repressed IFIT-1 induction by rIFN. We have thus provided evidence that the ability of neutralizing Abs to elicit an IFN-like response or to further enhance the effects of type I IFN is only observed for mAbs targeting IFN-α or IFN-β in this cellular context.

Because the response was inhibited by concomitant administration of IFNAR blocking Abs even in the absence of exogenously added rIFN, we hypothesized that ECs might constitutively express low levels of type I IFNs which might contribute to the effects observed. The constitutive low-level expression of type I IFN has previously been reported for other cell types (1, 2). When testing EC culture supernatants, IFN was not detectable at ELISA sensitivities of 4 U/ml (IFN-α) or 10 U/ml (IFN-β). However, considering that basal subthreshold IFN concentrations were reported around 0.1 U/ml (2, 3), ELISA sensitivity may have been limiting (data not shown). We therefore proceeded to block the potential constitutive production of type I IFN by siRNA application. The approach was limited to IFN-β because silencing of IFN-α genes is difficult to accomplish due to the variety of IFN-α subtypes that may be expressed. Furthermore, basal expression of IFN-α in fibroblasts was shown to be dependent on the constitutive IFN-β production, arguing for a predominant role of IFN-β in the low level, basal expression of type I IFNs (18). Comparably, we observed that the application of IFN-β siRNA led to the concurrent down-regulation of IFN-β and IFN-α subtypes generally expressed in ECs (19). Silencing of IFN-β expression in HDMECs was found to greatly reduce the IFN-like response to IFN blocking mAbs: IFIT-1 expression levels were reduced to ~40% irrespective of the Ab specificity to IFN-α or IFN-β, thus reflecting the impact of IFN-β siRNA on the overall type I IFN expression. We therefore propose that ECs maintain a basal level of IFN expression and IFNAR phosphorylation, which allows for their IFN-like response to IFN blocking mAbs. Because the constitutive, weak IFN signal is also known to be a prerequisite for the efficient cellular response to a high-level IFN challenge in, for example, mouse embryonic fibroblasts (3), we conducted a control experiment with 100 pg/ml rIFN-α or rIFN-β. IFIT-1 induction by high-dose IFN was similarly impaired by IFN-β silencing. These results further support our argument for an essential, basal IFN-β expression in primary ECs, which promotes their capacity for efficient and rapid cellular activation.

The further investigations focused on the potential involvement of Fc domains in the endothelial activation by IFN-neutralizing Abs. As the mAbs tested were of the mouse IgG1 isotype, a cross-reaction with human FcγRs seemed feasible. When comparing the full-length Ab with Fab or F(ab’)_2 fragments of an IFN-α blocking mAb, the fragments did not elicit an IFN-like response in ECs thus pointing to the importance of the Ab Fc domain. Furthermore, the fragments potently inhibited IFIT-1 induction by rIFN, i.e., they exhibited the expected IFN-neutralizing capacity and they could compete for the activity of the corresponding full-length Ab. The latter was, however, not as effective as the inhibition of rIFN. This effect might potentially relate to a better accessibility of intact Ab to autocrine IFN if the Ab was membrane-associated, or bound to Fc receptors. The observation that the EC response to intact anti-IFN mAb was also reduced in the presence of a mouse IgG1 isotype Ab (containing an Fc portion), further suggested involvement of Fc receptors. ECs are known to express FcγRs with an apparent heterogeneity depending on the vessel type. Various isoforms of FcγRII as well as FcγRI and neonatal Fc receptor have been detected on ECs with CD32 being the most prominent on HDMECs (20, 21). Yet, we could not demonstrate CD32 expression on our endothelial isolates nor block the effect of the anti-IFN Abs by concomitant treatment with a neutralizing Ab directed against CD32 (data not shown). However, these results do not exclude the potential involvement of an endothelial Fc receptor other than CD32.

Interestingly, Fc receptors have been localized to EC caveolar membrane sections, which sets them in close proximity to IFNAR molecules (3, 22). With respect to their interrelation, two settings may be envisioned. The mere local proximity of IFNAR and Fc receptors might serve to sequester autocrine IFN at the cell surface. IFN-neutralizing Abs on Fc receptors might thus increase the local type I IFN concentration beyond the signal threshold provided that IFN bound to the blocking mAbs can be released, i.e., passed onto IFNAR. Alternatively, a direct receptor interaction between Fc receptor and IFNAR could occur, initiated by the anti-IFN-α/β Abs and resulting in IFNAR activation beyond the basal “ready state”. Both players, IFNAR and FcγRs, have been reported to engage in diverse receptor cross-talk (2, 3, 23). Thus, whether Fc receptors are indeed involved in the IFN-like response to the neutralizing Abs or whether the Ab Fc domain mediates engagement of another, as yet unidentified cellular factor, is of prime interest for further investigations.

The activating potential of neutralizing IFN Abs was observed for all types of ECs tested and was not abolished by concomitant proinflammatory activation of ECs. In addition, the fact that IFN-α/β-neutralizing Abs did not only trigger an IFN-like response on quiescent cells but could also enhance the effect of a high-dose IFN challenge, emphasizes the potentially adverse systemic implications. The induction level of IFN response genes varied to some extent with primary EC isolates. When other cell types were investigated, a heterogeneous response was observed that may relate to Fc repertoire or differences in the potency of the signaling cascade. Interestingly, the IFN-like response to monoclonal-neutralizing Abs directed against human type I IFN was also recorded for human PBMCs. Although this observation extends the potential clinical impact these Ab effects might have, it seems intriguing why these effects have not been noted previously by other groups in comparable experiments on leukocytes. In preliminary investigations, we have gathered an indication pointing to the importance of the Ab type. Although all the Abs presented in this study were monoclonals of the mouse IgG1 isotype, we did not find an
IFN-like response to rabbit polyclonal anti-IFN antiserum in our experimental setting (data not shown). Thus, the nature and isotype of the Ab may be a crucial determinant.

The clinical application of recombinant mAbs targeted at IFN-\(\alpha\) is at the current therapeutic focus of autoimmune diseases. In light of the recent launch of the first clinical trial testing an IFN-\(\alpha\)-neutralizing mAb for the treatment of systemic lupus erythematosus, a possibly pleiotropic Ab effect would seem of particular concern. Our results would suggest that enhanced neutralizing efficiency might be achievable by testing Fab or F(ab\(^\prime\))\(_2\) fragments vs full-length Igs in systemic settings.

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Disclosures

The authors have no financial conflict of interest.

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ENDOTHELIAL ACTIVATION BY IFN Blocking Abs