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Intravenous Immunoglobulin Promotes Antitumor Responses by Modulating Macrophage Polarization

Ana´lges Domı´nguez-Soto,†*1 Mateo de las Casas-Engel,**† Rafael Bragado,† José Medina-Echezey,‡ Laura Aragoneses-Fenoll,* Enrique Martı´n-Gayo,§ Nico van Rooijen,† Pedro Berraondo,† María L. Toribio,† María A. Moro,¶ Isabel Cuartero,‖ Antonio Castrillo,¶¶ David Sancho,‡‡ Carmen Sánchez-Torres,‡‡ Pierre Bruhns,¶¶¶ Silvia Sánchez-Ramón,¶¶¶ and Angel L. Corbi*

Intravenous Igs (IVIg) therapy is widely used as an immunomodulatory strategy in inflammatory pathologies and is suggested to promote cancer regression. Because progression of tumors depends on their ability to redirect the polarization state of tumor-associated macrophages (from M1/immunogenic/proinflammatory to M2/anti-inflammatory), we have evaluated whether IVIg limits tumor progression and dissemination through modulation of macrophage polarization. In vitro, IVIg inhibited proinflammatory cytokine production from M1 macrophages and induced a M2-to-M1 polarization switch on human and murine M2 macrophages. In vivo, IVIg modified the polarization of tumor-associated myeloid cells in a FcγR1γ chain–dependent manner, modulated cytokine blood levels in tumor-bearing animals, and impaired tumor progression via FcγRIII (CD16), FcγRIV, and FcγRγR engagement, the latter two effects being macrophage mediated. Therefore, IVIg immunomodulatory activity is dependent on the polarization state of the responding macrophages, and its ability to trigger a M2-to-M1 macrophage polarization switch might be therapeutically useful in cancer, in which proinflammatory or immunogenic functions should be promoted. The Journal of Immunology, 2014, 193: 000–000.

Macrophages exhibit a huge functional plasticity and can acquire a continuum of polarization states in response to endogenous and nonself stimuli (1–4). Microbe-derived factors, or cytokines like IFN-γ, GM-CSF, or TNF-α, promote in macrophages the acquisition of proinflammatory, bactericidal, tumor-suppressive, and immunogenic activities, a process commonly referred to as classical or M1 polarization and whose hallmark is the ability to release large amounts of IL-12/IL-23 (2). Conversely, cytokines such as IL-4, IL-10, TGF-β, or M-CSF promote anti-inflammatory, scavenging, tumor-promoting, tissue repair, and proangiogenic functions, all of which are grouped under the terms “alternative” or M2 polarization, which endows them with the ability to produce high levels of IL-10 (3–5). M1-polarized macrophages predominate at the initial stages of an inflammatory response, whereas M2-type macrophages drive resolution of inflammation and tissue repair after injury and maintain tissue homeostasis (6). In vivo, the misbalance of macrophage polarization states underlies numerous pathophysiological processes, including tumor development, autoimmune diseases, and chronic inflammatory pathologies (6–8).

The switch between M1 and M2 polarization states is especially relevant in tumor initiation, progression, and dissemination, which are extremely reliant on the presence and polarization state of macrophages within the tumor stroma (tumor-associated macrophages [TAM]) (5). The contribution of macrophages to tumor development is inferred from the poor outcome associated with enhanced levels of M-CSF, the reduced metastasis observed in Csf1ropop mice (9, 10), and the positive correlation between high content of TAM and a bad prognosis (11). Depending on their polarization status, macrophages can either promote antitumor

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*Centro de Investigaciones Biológicas/Consejo Superior de Investigaciones Científicas, Madrid 28040, Spain; †Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Madrid 28040, Spain; ‡Center for Applied Medical Research, Pamplona 31009, Spain; §Centro de Biología Molecular, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid 28049, Spain; ¶Department of Molecular Cell Biology, Free University Medical Center, Amsterdam 1081, the Netherlands; ‡Unidad de Investigación Neurovascular, Departamento de Farmacología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid 28040, Spain; ††Centro de Investigaciones Biomédicas Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid 28029, Spain; ‡‡Centro de Investigaciones Biomédicas-Asociación de Biomedicina, Consejo Superior de Investigaciones Científicas-Universidad de Las Palmas de Gran Canarias 35001, Spain; †††Fundación Centro Nacional de Investigaciones Cardiovasculares, Centro Nacional de Investigaciones Cardiovasculares, Madrid 28029, Spain; ‡‡‡Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 07360, Mexico; †††Institut Pasteur, Département d’ImmunoLOGie, Unité des Anticorps en Thérapie et Pathologie, Paris 75015, France; ††‖INSERM U760, Paris 75015, France; and †‖Hospital General Universitario Gregorio Marañón, Madrid 28007, Spain

†A.D.-S. and M.d.l.C.-E. contributed equally to this work, and the order of authors should be considered arbitrary.

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Address correspondence and reprint requests to Prof. Angeles Domínguez-Soto and Prof. Angel L. Corbi, Centro de Investigaciones Biológicas/Consejo Superior de Investigaciones Científicas, Ramiro de Maeztu, 9, 28040 Madrid, Spain. E-mail addresses: ads@cib.csic.es (A.D.-S.) and acorbi@cib.csic.es (A.L.C.).

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Abbreviations used in this article: BMDM, bone marrow–derived macrophage; IVIg, intravenous Ig; MCA, middle cerebral artery; TAM, tumor-associated macrophage; WT, wild-type.

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immune responses or contribute to tumor progression (12). In fact, as tumor progresses, TAM develop an immunosuppressive and protumoral phenotype, which fuels tumor growth, metastasis, and suppression of tumor-specific immune responses (13).

Intravenous Ig (IVIg) is a preparation of polyclonal and polyclonal IgGs derived from the plasma of thousands of healthy donors. IVIg therapy is Food and Drug Administration approved for primary immunodeficiencies, immune thrombocytopenic purpura, and Kawasaki's disease, and is beneficial for multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (14, 15). Previous reports have evidenced that IVIg exerts potent immunomodulatory actions in immunodeficiency syndromes, autoimmune diseases, and infectious processes (16). The molecular and cellular basis for the IVIg immunomodulatory action, including the identity of the biologically active constituents in IVIg and its specific cell surface receptors and target cells, remains to be completely clarified (17–20). In the present report, we demonstrate that IVIg promotes a M2-to-M1 macrophage polarization switch through ligation of activating Fc receptors in both human and mouse macrophages, and that FcγRIII, FcγRI, and the FcγR chain mediate the IVIg-induced repolarization of tumor-associated myeloid cells and inhibition of tumor progression and metastasis in vivo.

Materials and Methods

Macrophage differentiation, cell culture, and treatments

Human PBMC isolation was carried out, as described previously (21). Human TAM were obtained from the pleural fluid of a metastatic breast adenocarcinoma patient, after obtaining written informed consent, following Medical Ethics Committee procedures (Hospital General Universitario Gregorio Marañón), and using CD14 microbeads, as described (22). Human postnatal thymocytes were isolated from thymus fragments removed during corrective cardiac surgery of patients aged 1 mo to 4 y, after providing informed consent in accordance with the Declaration of Helsinki. Thymocyte cell suspensions were enriched in non-T cells by a non-T cells by sheep erythrocyte resetting, as previously described (23). Intrathymic macrophages (≥95% CD13+ CD11c+CD14+) were obtained from the resulting cell fraction by positive selection using a PE-labeled anti-CD14 mAb and anti-PE microbeads (Miltenyi Biotec). Bone marrow–derived macrophages (BMDM) were obtained, as described previously (24, 25). For activation, macrophages were treated with Escherichia coli 055:B5 LPS (100 ng/ml for mouse macrophages; 10 ng/ml for human macrophages) for 24 h. The B16F10 mouse melanoma cell line (C57BL/6 origin), the mouse MC38 colon carcinoma cell line, and the highly invasive human BLM melanoma cell line (provided by J. Teixido, Centro de Investigaciones Biológicas/Consejo Superior de Investigaciones Científicas) were maintained in RPMI 1640 (MC38) or DMEM (BLM, B16F10) medium supplemented with 10% FCS, at 37°C, in a humidified atmosphere with 5% CO2. Fully polarized macrophages were exposed to 10 mg/ml IVIg (Priven; CSL Behring) for 24 h. For inhibition assays, differentiated macrophages were treated with vehicle (H2O) or piceatannol (100 μM; Calbiochem) 1 h before IVIg treatment. To determine the CD16 contribution, an anti-human CD16 F(ab)2 mAb (LSBio, clone 3G8), or an isotype-control mouse IgG1 F(ab)2, was used at 20 μg/ml before IVIg treatment.

Quantitative real-time PCR

Total RNA was extracted using the RNeasy Mini kit or AllPrep DNA/RNA/Protein Mini kit (Qiagen) following manufacturer’s guidelines. cDNA was synthesized using the Reverse Transcription System kit (Applied Bio-systems). Oligonucleotides for selected genes were designed according to the Roche Universal Probe library (Roche Applied Science). Quantitative real-time PCR was performed using custom-made panels (Roche Diagnostics) or standard plates on a LightCycler 480 (Roche Diagnostics) or a iQTM5 (Bio-Rad), respectively. An extensive battery of genes differentially expressed between M1 and M2 macrophages was included in our assays (a total of 33 genes, of which 13 were previously identified as M1 marker genes and 20 as M2 marker genes) (21, 26, 27). Assays were made in triplicate, and results were normalized according to the expression level of GAPDH or to the mean of the expression level of endogenous reference genes Hprt, Sdha, and Tbp. Results were expressed using the ΔΔ cycle threshold method for quantitation.

ELISA

Culture supernatants from LPS-treated (24-h) human macrophages were assayed for the presence of cytokines using commercially available ELISA for TNF-α, IL-10, IL-6 (ImmunoTools), IL-12p40, CCL-2 (OpeEIATM IL-12 p40 set; BD Pharmingen), and activin A (R&D Systems). LPS-treated mouse macrophage supernatants were tested for IL-10, TNF-α, and CCL-2 using available ELISA (BioLegend). ELISA was performed following the protocols supplied by the manufacturers.

Cell proliferation assays

BLM cells were plated (5 × 104 cells/well), allowed to adhere for 24 h, and exposed to culture supernatants from untreated or IVIg-treated human macrophages for 48 h. Cell proliferation was evaluated using MTT (Sigma-Aldrich). Complete media was used as control to determine the basal BLM cell proliferation.

Mouse tumor models

For the pulmonary metastasis animal model, 6- to 8-wk-old wild-type (WT) C57BL/6, Fcgr3−/−, and Fcrlg−/− mice (B6.129P2-Fcgr3m1Rep/J) from the Jackson Laboratory, provided by J. Ochando and S.-H. Chen, Mount Sinai School of Medicine, New York, NY, were used for all experiments. Mice were injected i.v. (tail vein) with 3 × 105 B16F10 melanoma cells in 0.1 ml sterile PBS on day 0, and with 10 mg/ml IVIg (100 mg/ml) or PBS (control) 24 h before tumor cell injection (day −1) and on days 6 and 13. Mice were sacrificed on day 18, and lung surface metastases were counted under a dissecting microscope as black nodules after bleaching in Fekete’s solution. For xenograft studies, 2- to 3-mo-old BALB/c SCID, C57BL/6, Fcgr3−/−, and Fcrlg−/− (generated by J. Ravetch and bred at Institute Pasteur), and Fcrlg−/− mice were injected s.c. (lateral thoracic wall) with 2 × 106 BLM cells or 5 × 105 MC38 cells in 0.1 ml PBS (PBS) or PBS (400 μl) was injected i.v. on days −1, 7, and 14, and mice were killed 17–21 d after cell tumor inoculation. Mice were inspected daily, and the tumor volume was measured as width2 × length/2. All protocols were approved by the Centro de Investigaciones Biológicas/Consejo Superior de Investigaciones Científicas Ethics Committee.

Permanent middle cerebral artery occlusion in mice

The surgical procedure was a variant of that described by Chen et al. (28) and Liu et al. (29). Mice were anesthetized with isoflurane 1.5–2% in a mixture of 80% air/20% oxygen, and body temperature was maintained at physiological levels with a heating pad during the surgical procedure and anesthesia recovery. Mice were subjected to permanent focal cerebral ischemia through the distal occlusion of middle cerebral artery (MCA) by ligation of the trunk just before its bifurcation between the frontal and parietal branches with a 9-0 suture, in combination with the occlusion of the ipsilateral common carotid artery. Physiological parameters were not significantly different among the different groups studied. Following surgery, individual animals were returned to their cages with free access to water and food. All the groups were performed and quantified in a randomized fashion by investigators blinded to treatment groups. IVIg (400 μl, 100 mg/ml) or PBS (400 μl) was injected by vein tail 10 min after MCA occlusion. Forty-eight hours afterward, permanent MCA occlusion mice were killed by an overdose of sodium pentobarbital and brain was removed, cut into 1-mm-thick coronal slices, and stained with 2,3,5-triphenyl tetrazolium chloride (1% in 0.1 M phosphate buffer). Infarct volumes were calculated sampling each side of the coronal sections with a digital camera (Nikon Coolpix 990), and the images were analyzed using ImageJ 1.33u (National Institutes of Health, Bethesda, MD). To exclude the brain edema effects, infarct area was corrected by the ratio of the entire area of the ipsilateral hemisphere to that of the contralateral.

LPS-primed endotoxin shock model mice

Mice received i.p. injections of 350 μg LPS per 25 g body weight 1 h after injection of 400 μl IVIg (100 mg/ml) or PBS. LPS was dissolved prior to injection in PBS at a concentration of 10 mg/ml. Injected animals were monitored for 7 d.

Statistical analysis

Differences between the experimental groups in in vivo experiments were analyzed by a nonpaired Student t test. In the case of CD11b+ isolated cells, quantitative PCR data were analyzed with the REST-2009 software from Qiagen using 5000 permutations. Statistical significance of in vitro generated data was evaluated using a paired Student t test. In all cases, p < 0.05 was considered as statistically significant.
Results

**M1 and M2 macrophages are differentially affected by IVIg**

Because IVIg is used off-label for chronic inflammatory diseases (30), in which M1 macrophages critically contribute to pathology, we first investigated whether IVIg modulates the effector functions of proinflammatory M1 macrophages. A 24-h exposure to IVIg led to a significant reduction in the LPS-stimulated release of TNF-α, IL-12p40, and CCL2 from M1 macrophages (Fig. 1A), without affecting their tumor cell growth-inhibitory ability (Fig. 1C, upper panel). IVIg did not overtly alter the gene expression profile of M1 macrophages, as most M1 polarization-specific markers were only weakly modulated in response to the treatment (Supplemental Fig. 1A). The functional modulation induced by IVIg on M1 macrophages is, therefore, compatible with the previously reported anti-inflammatory activity of IVIg and might help explain the clinical improvement of chronic inflammatory diseases by IVIg treatment (14, 17, 19, 31).

The effects of IVIg on macrophages under homeostatic conditions, or in M2-associated pathophysiological processes, had not been addressed before. Unlike M1 macrophages, treatment of M2-polarized macrophages with IVIg for 24 h led to a significant reduction in the LPS-induced CCL2 and IL-10 release and a concomitant enhancement of the LPS-induced production of TNF-α and IL-12p40 (Fig. 1B). Moreover, IVIg-treated M2 macrophage supernatants, like M1 macrophage-conditioned media, inhibited the growth of BLM melanoma cells (Fig. 1C, lower panel), whose proliferation was not affected by IVIg itself. Along the same line, IVIg provoked a dramatic transcriptomic switch in M2 macrophages, as it reduced the expression of M2-specific markers (between 5 and 100 times) and increased the expression of M1-specific markers (10- to 100-fold) (Fig. 1D). Kinetic analysis revealed that the polarization switch of M2 macrophages is already evident 12 h after IVIg addition (Supplemental Fig. 1B). Unsupervised hierarchical clustering confirmed that the gene expression profile of IVIg-treated M2 macrophages resembles that of proinflammatory M1 macrophages (Fig. 1E). These results indicated that IVIg differentially affects the functional and transcriptomic polarization of M1 and M2 macrophages, as it inhibits the pro-inflammatori...
duction of proinflammatory cytokines by M1 macrophages while promoting the acquisition of a proinflammatory profile in M2 macrophages. Further supporting these results, IVIg increased the expression of most M1-specific markers, downregulated the majority of M2-specific markers on ex vivo isolated CD14+ human thymic macrophages (Supplemental Fig. 2A), and reduced the constitutive and LPS-induced expression of IL-10, while potentiating that of TNF-α, from CD14+ tumor-associated macrophages (Supplemental Fig. 2B). Altogether, these results demonstrate the ability of IVIg to promote a M2-to-M1 polarization switch in human macrophages.

**CD16 and Syk phosphorylation mediates the IVIg-induced switch in macrophage polarization**

To unravel the mechanisms underlying the IVIg effect on human macrophage polarization, we initially focused on activating Fcγ receptors, especially on CD16, whose expression is significantly higher in M2 than in M1 macrophages (Fig. 2A, 2B). The contribution of the CD16-encoding FCGR3A gene to the IVIg-mediated functional polarization switch was assessed by using the anti-CD16 3G8 blocking Ab. The 3G8 F(ab')2 significantly reversed the IVIg-mediated change in cytokine profile, as it abrogated the IVIg-dependent increase in TNF-α production and inhibited the IVIg-mediated reduction in IL-10 release (Fig. 2C). Moreover, anti-CD16 3G8 F(ab')2 impaired the IVIg-induced downregulation of M2-specific marker expression, including that of IL10, as well as the increase of M1-specific markers (Fig. 2D). Taken together, these results suggest that CD16 is involved in the M2-to-M1 phenotypic and functional polarization switch induced by IVIg. In line with the known CD16-dependent Syk phosphorylation, IVIg triggered activation of Syk and its downstream targets Akt, ERK1/2, CREB, and p38MAPK (Supplemental Fig. 3A) (32, 33), but had no effect on the phosphorylation state of SHIP1 (our unpublished observation), a readout for the engagement of the inhibitory CD32B Fcγ receptor (31) (Fig. 2E, Supplemental Fig. 3C, 3D). The IVIg-induced polarization switch was affected by the Syk tyrosine kinase inhibitor piceatannol (Fig. 2E, Supplemental Fig. 3C, 3D), although some of its effects were distinct (e.g., IL10) to those seen in the presence of the blocking anti-CD16 Ab. These results indicate that both CD16 and Syk contribute to the M2-to-M1 macrophage polarization switch induced by IVIg in human macrophages, and that other activating Fc receptors, and not only CD16, might mediate the effect of IVIg on IL10 gene expression.

**IVIg modifies bone marrow–derived M2 mouse macrophage polarization via FcγR-chain, FcγRIV, and FcγRIII**

To determine the extent of the relevance of these findings, we next determined whether the IVIg-mediated polarization switch was...
also observed in mouse BMDM. Like in the case of human macrophages, a 24-h treatment with IVIg significantly enhanced the expression of the paradigmatic M1 markers Nos2, Tnfa, and Cd11c, whereas it inhibited the expression of a large number of M2 polarization markers (Fig. 3A). At the functional level, the LPS-stimulated production of TNF-\(\alpha\) by mouse M2 BMDM was significantly enhanced by IVIg, without affecting IL-10 release (Fig. 3B). This IVIg-induced polarization switch took place via Fc receptors, because 1) deletion of the Fcgr3 gene (encoding FcγRIII) inhibited the increase of Nos2 and the decrease of Cbr2, Emr1, and Cd206 triggered by IVIg (Fig. 3A); 2) deletion of the Fcgr4 gene (encoding FcγRIV) inhibited the IVIg-mediated decrease in Hpdg, Cla2b, Gas6, Stab1, Cbr2, Emr1, Mgl1, and Cd206 (Fig. 3A); and 3) all the IVIg-triggered gene expression changes were blunted in Fcer1g\(^{2/2}\) macrophages (Fig. 3A). Regarding LPS-cytokine release, the absence of FcRγ-chain or FcγRIV expression completely abolished the IVIg-induced increase in TNF-\(\alpha\) release from mouse M2 BMDM (Fig. 3B). Consequently, all the transcriptomic and functional changes triggered by IVIg on M2 BMDM are absent in Fcer1g\(^{2/2}\) macrophages, whereas ablation of the Fcgr3 or Fcgr4 genes has a partial influence on the polarization switch triggered by IVIg. Interestingly, the impairment of the IVIg-induced M2-to-M1 polarization switch in Fcer1g\(^{2/2}\) macrophages might be explained by their skewed basal M2 polarization, illustrated in Supplemental Fig. 4 and also recently reported (34), and that might make Fcer1g\(^{2/2}\) macrophages more reluctant to the polarizing effect of IVIg. Therefore, and as in the case of human macrophages, Fcγ-activating receptors mediate the proinflammatory polarization of mouse macrophages by IVIg.

**IVIg promotes proinflammatory response in vivo**

To assess the in vivo relevance of the above in vitro data, we evaluated the influence of IVIg on three animal models of
disease, as follows: a MCA occlusion stroke and LPS-induced sepsis-like mouse models, in which tissue damage correlates with excessive proinflammatory responses (35), and a xenograft tumor model, in which tumor and tumor-associated cells contribute to the establishment of an immunosuppressive environment (36). IVIg injection led to significant increases in the volume of the infarcted area in the stroke mouse model (Fig. 4A) and diminished survival rates after injection of a lethal dose of LPS (Fig. 4B), thus implying that IVIg misbalances innate immune responses toward a more proinflammatory state. Along the same line, and regarding the xenograft tumor model, IVIg significantly reduced tumor volumes after 15 d (Fig. 4C). Altogether, data from the three assayed animal models revealed that IVIg exerts a global proinflammatory response in vivo.

**FIGURE 4.** Effect of IVIg on different animal models: infarct outcome after permanent MCA occlusion, LPS-induced endotoxin shock, and s.c. tumors. (A) Infarct volume (left panel) and infarct areas (right panel) assessed by 2,3,5-triphenyl tetrazolium chloride staining 48 h after permanent middle cerebral artery occlusion in brains from IVIg-treated or untreated mice (n = 10 in each case). The mean ± SEM is indicated (**p < 0.05**). (B) C57BL/6 mice were pretreated i.p. with IVIg or PBS and 1 h after injected i.p. with 350 μg LPS. The survival was monitored during 7 d. Data represent mean of pool from n = 20 mice. (C) Effect of i.v. injection of IVIg on the volume of tumors found after s.c. injection of BLM human melanoma cells in the lateral thoracic wall of BALB/c SCID mice. The mean ± SEM is indicated. Date are pool from n = 8 mice (**p < 0.05, ***p < 0.01).

**Inhibition of mouse tumor metastasis by IVIg is associated with changes in macrophage polarization and depends on Fcγ receptors**

Because tumor metastasis and progression are dependent on the tumor ability to alter macrophage polarization (5, 37) and given the above described effects of IVIg, we hypothesized that IVIg might inhibit tumor growth and/or metastasis by skewing macrophage polarization via engagement of Fcγ receptors. In the MC38 colon cancer xenograft model, IVIg significantly reduced tumor volume in WT (Fig. 5A) and *Fcer1g*−/− mice (Fig. 5B), but had no effect in *Fcgr3*−/− (Fig. 5C) or *Fcer1g*−/− *Fcer3*−/− mice (Fig. 5D), indicating that the latter two genes contribute to the antitumor activity of IVIg. Besides, IVIg significantly lowered B16F10 melanoma lung metastasis in WT mice, an inhibitory effect that was absent in *Fcer3*−/− and *Fcer1g*−/− mice (Fig. 5E). Importantly, analysis of the tumor-associated CD11b+ myeloid cells from MC38 tumor xenografts revealed a significantly increased expression of the M1 polarization-associated markers *Cd11c*, *Ccr7*, and *Nos2* in tumor-bearing WT mice, but not in *Fcgr3*−/− mice (Fig. 5F). Even more, the IVIg-mediated global upregulation of M1-specific markers group seen in WT mice was completely abolished in *Fcgr3*−/− mice (Fig. 5F). Therefore, and through engagement of activating Fcγ receptors, IVIg treatment impairs tumor progression (growth and metastasis) and influences the polarization of tumor-associated myeloid cells. The causal relationship between both effects was assessed by determining the ability of IVIg to inhibit tumor growth upon macrophage depletion. As shown in Fig. 5G, clodronate liposome-mediated depletion of macrophages prevented the IVIg-promoted tumor growth reduction in the MC38 colon cancer model. In fact, even the decrease in serum CCL2 caused by IVIg treatment was found to be macrophage dependent (Fig. 5H). Therefore, IVIg alters the polarization of macrophages, whose presence is absolutely required for IVIg to limit tumor growth.

**Discussion**

The immunomodulatory action of IVIg has widened the range of pathologies for which IVIg therapy is either approved or has shown benefit (38, 39). In line with its beneficial actions on inflammatory pathologies, we now show that IVIg impairs the effector functions of proinflammatory M1 macrophages. However, we also report the ability of IVIg to cause a M2-to-M1 phenotypic and functional Fc receptor-mediated polarization switch on human and murine macrophages in vitro and in vivo, thus illustrating that the IVIg immunomodulatory effects are dependent on the polarization state of the responding macrophages.

Immunocomplexes have long been known to promote tumor cell killing in a FcγR-dependent manner and to elicit potent inflammatory responses that underlie pathologies such as systemic lupus erythematosus and rheumatoid arthritis (40). From this point of view, the proinflammatory and antitumor nature of the IVIg effect that we report is not unprecedented. Paradoxically, however, high doses of IgG (IVIg) exert beneficial effects on several autoimmune disorders by virtue of their potent anti-inflammatory activity (16, 41). Attempts to explain this apparent contradiction have indicated that the active components within IVIg consist of a minor fraction of the preparation (e.g., immune complexes, sialylated Fc IgG), thus explaining the large doses requirement (14). The results that we now present shed more light on this issue, because IVIg provokes different responses, either pro- or anti-inflammatory, depending on the polarization state of the target macrophage. Whereas IVIg improves inflammatory diseases through impairment of the functional activities of M1 proinflammatory...
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FIGURE 5. IVIg inhibition of tumor progression requires macrophages and is dependent on FcγRIII, FcγRIV, and FcγR-chain. Tumor volume in C57BL/6 mice (A), Fcγ3−/− (B), Fcγ1−/− (C), or Fcγ4−/− (D) that had been s.c. injected with MC38 colon cancer cells in the lateral thoracic wall and i.v. injected with either PBS (control) or IVIg. Shown are the mean ± SEM [n = 7 in (A)–(C), n = 5 in (D)]; *p < 0.05. (E) Number of lung metastatic foci in PBS (−) or IVIg-treated WT (n = 6), Fcγ3−/− (n = 6), and Fcγ1−/− (n = 11) C57BL/6 mice i.v. injected with B16F10 melanoma cells. Shown are the mean ± SEM (*p < 0.05). (F) Polarization marker expression in CD11b+ cells isolated from tumors in PBS (control) or IVIg-treated (24 h) WT and Fcγ1−/− mice s.c. injected with MC38 colon cancer cells. In all cases, the ratio of the expression level of each gene in tumor-associated CD11b+ in IVIg- versus PBS-treated animals (IVIg/control) is represented (*p < 0.05). The comparison between the global expression of M1-specific markers in WT and Fcγ1−/− mice is also shown (*p < 0.05). (G) Tumor volume in PBS (control) or IVIg-treated WT C57BL/6 mice s.c. injected with MC38 colon cancer cells in the lateral thoracic wall, either with or without clodronate liposome-mediated depletion of macrophages. Shown are the mean ± SD (n = 8) (control versus IVIg-treated mice: p < 0.05, **p < 0.01; control versus control + clodronate: **p < 0.05, ***p < 0.01; IVIg versus IVIg + clodronate: *p < 0.05). (H) Serum cytokine levels in WT mice, PBS (−) or IVIg treated, either with or without clodronate liposome-mediated depletion of macrophages. Shown are the mean ± SD (n = 8; *p < 0.05, **p < 0.01).

In the context of cancer, we have demonstrated that IVIg impairs tumor progression and metastasis in a Fc receptor- and macrophage-dependent manner, and that IVIg alters the expression of polarization markers in CD11b+ tumor-associated myeloid cells from WT but not Fcγ1−/− mice. The coexistence of autoimmune pathologies and cancer has previously provided evidence that IVIg therapy promotes regression of cancer in patients with chronic lymphatic leukemia, Kaposi’s sarcoma, and melanoma (42). Given the importance of the polarization of TAM for tumor progression and dissemination (37), our results establish a sequential link between the IVIg ability to modulate macrophage polarization and its antitumor effect, suggesting that the proinflammatory activities of IVIg might also be therapeutically useful in pathologies.
such as cancer, in which immunogenic and proinflammatory macrophage functions need to be promoted.

Numerous molecular and cellular mechanisms have been proposed to explain the immunomodulatory activity of IVIg (19). Our results clearly establish macrophages as an absolute requirement for the antitumor effect of IVIg, because macrophage depletion abrogates the inhibition of tumor growth by IVIg. Moreover, in tumor-bearing animals, IVIg inhibited the level of circulating Ccl2, whose tumor-dependent increase was significantly reduced upon clodronate-mediated macrophage depletion. This is of particular significance because CCL2 is known to promote M2 tumor-associated macrophages in tumor-bearing animals (43, 44). CCL2-induced MCPIP1 expression of the M2-associated IL-10–driving maf transcription factor (26, 46). The ability of IVIg to reduce CCL2 both in vivo (Fig. 5H) and in vitro (Fig. 1A, 1B) might, therefore, contribute to lower the M2/anti-inflammatory environment seen in tumor-bearing animals, thus favoring the generation of antitumor responses. In this regard, others have also shown that IVIg decreases CCL2 levels in whole blood (47) and skin tissue (48).

Besides monocytes/macrophages, cellular targets for IVIg include NK cells, Tand B lymphocytes, granulocytes, and endothelial cells (49). Our data also support the idea that cells other than macrophages contribute to the proinflammatory activity of IVIg because the IVIg-induced increase in circulating TNF-α in tumor-bearing animals is not eliminated upon macrophage depletion (Fig. 5H). Because inhibition of tumor growth by IVIg is macrophage dependent, it seems reasonable to conclude that the IVIg-enhanced levels of TNF-α do not significantly participate in the antitumor action of IVIg. However, and given the TNF-α functional activities, it could be hypothesized that IVIg-induced TNF-α might explain the mild/adverse side effects occasionally seen during IVIg therapy.

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Disclosures

The authors have no financial conflicts of interest.

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