

Priority Brief

Transient Complement Inhibition Promotes a Tumor-Specific Immune Response through the Implication of Natural Killer CellsValérie Janelle^{1,2}, Marie-Pierre Langlois¹, Esther Tarrab¹, Pascal Lapierre¹, Laurent Poliquin², and Alain Lamarre^{1,2}**Abstract**

Although the role of the complement system in cancer development has been studied, its involvement in the development of an antitumoral immune response remains poorly understood. Using cobra venom factor (CVF) to inhibit the complement cascade via C3 molecule exhaustion in immunocompetent mice bearing B16gp33 melanoma tumors, we show that transient inhibition of the complement system allowed for the development of a more robust gp33-specific antitumoral CD8⁺ T-cell response. This immune response proved to be natural killer (NK) dependent, suggesting an interaction of complement proteins with this cellular subset leading to T lymphocyte activation and enhanced cytotoxic T-cell activity against tumor cells. This study demonstrates for the first time the implication of the complement system in the development of NK-mediated cytotoxic T-cell-dependent antitumoral immune responses. The complement pathway could therefore be a potent therapeutic target to improve NK-dependent antitumoral immune responses in patients with cancer. *Cancer Immunol Res*; 2(3); 200–6. ©2013 AACR.

Introduction

Historically, the complement system has been considered a "complementing" player in the innate immune response that mediated clearance of various pathogens or dead cells and could induce inflammation (1). In cancer, activated complement proteins were primarily described for their role in tumor defense either through complement-dependent cytotoxicity (2) or through antibody-dependent cell-mediated cytotoxicity (3). However, the complement anaphylatoxins C3a and C5a can increase the activation of phosphatidylinositol 3-kinase, Akt, and mTOR (4); when overexpressed, these proteins are strongly associated with neoplasia, suggesting a more complex role for the complement proteins in tumorigenicity. Furthermore, mice deficient in C3 or C4 show significantly decreased proliferation of TC-1 subcutaneous tumors compared with that in wild-type mice (5), whereas the C5a protein could be involved in CD8⁺ T-cell inhibition (6).

Natural killer (NK) cells are large granular lymphocytes that are part of the innate immune system implicated in host defense against tumors and pathogens (7). NK cells express FcγR, which interacts with immunoglobulin bound to antigen on target cells, leading to NK cell activation (8) and production of IFN-γ and TNF-α (9). NK cells can also complement CTLs by killing MHC class I-deficient cells that failed to be recognized by T cells (7). NK cells have been shown to cross talk with dendritic cells; their effector functions are stimulated through direct contact with activated dendritic cells (10). However, dendritic cell/NK cell interactions are bidirectional, resulting not only in NK cell activation but also dendritic cell maturation depending on the activation status of both cells (11). Soluble factors such as TNF-α and IFN-γ, as well as cell-to-cell contact, are required for NK-mediated dendritic cell activation (12).

Little is known about the link between the complement system and NK cells, but evidence suggests that complement receptor CR3, implicated in phagocytosis mediated by complement fragment iC3b-opsonized targets (13), is expressed by NK cells and cytotoxic T cells and displayed similar functions as CR3 expressed on phagocytes (14). Given that NK cells can be involved in tumor control and that proteins from the complement pathway may interact with NK cells to suppress or limit their functions, we hypothesized that proteins in the complement pathway may favor tumor growth through the impediment of NK cells. Herein, we found that a transient inhibition of the complement system increased NK cell proportions in the spleen and in the tumor, leading to the induction of an increased antitumoral CTL response.

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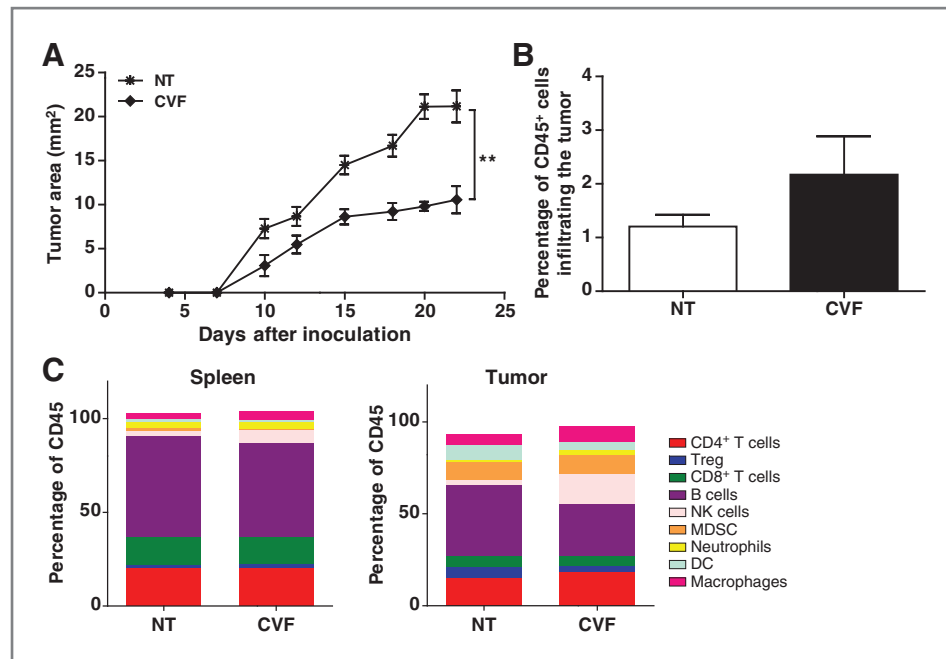
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Figure 1. Effect of CVF treatment on B16 tumor growth and immune cell infiltration. C57BL/6 mice were injected s.c. with B16 (day 0) and CVF was administered by i.p. injection on day 6. A, tumor growth was measured at indicated time points. Data show the mean \pm SEM and are representative of two independent experiments. **, $P < 0.01$. B, 9 days following CVF treatment, tumors and spleens were harvested and total tumor CD45⁺ cell numbers were determined; C, immune cell subpopulation proportions of CD4⁺ T cells (CD4⁺CD25⁻), Tregs (CD4⁺CD25⁺), CD8⁺ T cells, B cells (B220⁺), NK (Nkp46⁺), MDSC (Gr1⁺CD11b⁺), neutrophils (Gr1⁺CD11b⁻), dendritic cells (Gr1⁻CD11b⁺CD11c⁺), and macrophages (Gr1⁻CD11b⁺F4/80⁺) were determined. DC, dendritic cells; MDSC, myeloid-derived suppressor cells; NT, not treated.



Material and Methods

Cell lines

Murine B16 and B16gp33 melanoma cells were obtained from Dr. A. Ochsenbein (Department of Clinical Research, University of Bern; Bern, Switzerland). The B16gp33 cells (H2-D^b) were derived from B16.F10 cells transfected with a DNA minigene encoding the immunodominant CD8⁺ T-cell epitope of the lymphocytic choriomeningitis virus (glycoprotein aa 33-41; ref. 15). Cells were grown in Dulbecco's Modified Eagle Medium (Life Technologies) supplemented with 10% FBS and 200 μ g/mL of G418 to select for the retention of the gp33 minigene. No additional test or validation was performed on the cell lines used.

ELISA

C3 molecule concentration was tested in mice sera at the indicated time points by ELISA. Briefly, blood was harvested and incubated at 37°C to coagulate. Samples were centrifuged and the serum fraction was used. Serial dilutions were added onto an immunoglobulin M-coated 96-well plate (5 μ g/mL; Sigma-Aldrich). Samples were incubated with mouse anti-C3 coupled to biotin (Cedarlane) followed with streptavidin-horseradish peroxidase (Southern Biotech). A solution of o-phenylenediamine dihydrochloride (Sigma-Aldrich) was then used for the detection of C3 molecules. Optical density was measured by spectrophotometry at 490 nm.

In vivo studies

All procedures were approved by the INRS Institutional Animal Care and Use Committee. To establish subcutaneous tumors, 5×10^5 B16 or B16gp33 cells in 100 μ L of PBS were injected into the flanks of C57BL/6 mice. Six days after tumor implantation, mice were injected with cobra venom factor (CVF; 20 μ g/mouse; Quidel) intraperitoneally. For NK deple-

tion experiments, 200 μ g of purified monoclonal anti-NK1.1 antibodies from the PK136 hybridoma, kindly provided by Dr. Suzanne Lemieux (Institut national de la recherche scientifique, INRS-Institut Armand-Frappier; Laval, Québec, Canada), were injected intraperitoneally 2 days before decomplementation (Supplementary Fig. S2).

Flow cytometry

The spleens and tumors were recovered from mice and dissociated *in vitro* to achieve single-cell suspensions using nylon 100 μ m cell strainers (BD Falcon). Cells were washed, resuspended in PBS containing 1% bovine serum albumin and 0.1% sodium azide (fluorescence-activated cell sorting, FACS, buffer), and incubated with directly conjugated primary antibodies for 30 minutes at 4°C. Cells were then washed and resuspended in 200 μ L of FACS buffer containing 1% paraformaldehyde. Samples were acquired on a FACS Fortessa (BD Biosciences) and analyzed using the Flowjo software (Tree Star). Anti-CD45 phycoerythrin (PE)/CF-594, anti-CD25 allophycocyanin (APC), and anti-NKp46 Alexa 700 were purchased from BD Biosciences. Anti-CD4 APC/Cy7, anti-CD4 FITC, anti-CD8 PE/Cy7, anti-CD62L Alexa 700, anti-CD44 PercP/Cy5.5, anti-B220 APC/Cy7, anti-CD11b Pacific Blue, anti-Gr1 PE/Cy5, anti-CD11c FITC, and anti-F4/80 APC were purchased from BioLegend.

Dendritic cell activation

Single-cell suspensions were prepared from the spleens and draining lymph nodes harvested 9 days after CVF injection. Red blood cells were lysed and the remaining cells were resuspended in FACS buffer before CD11c-FITC and CD86-PE labeling (BioLegend). Samples were acquired on a FACS Fortessa (BD Biosciences) and analyzed using the Flowjo software (Tree Star).

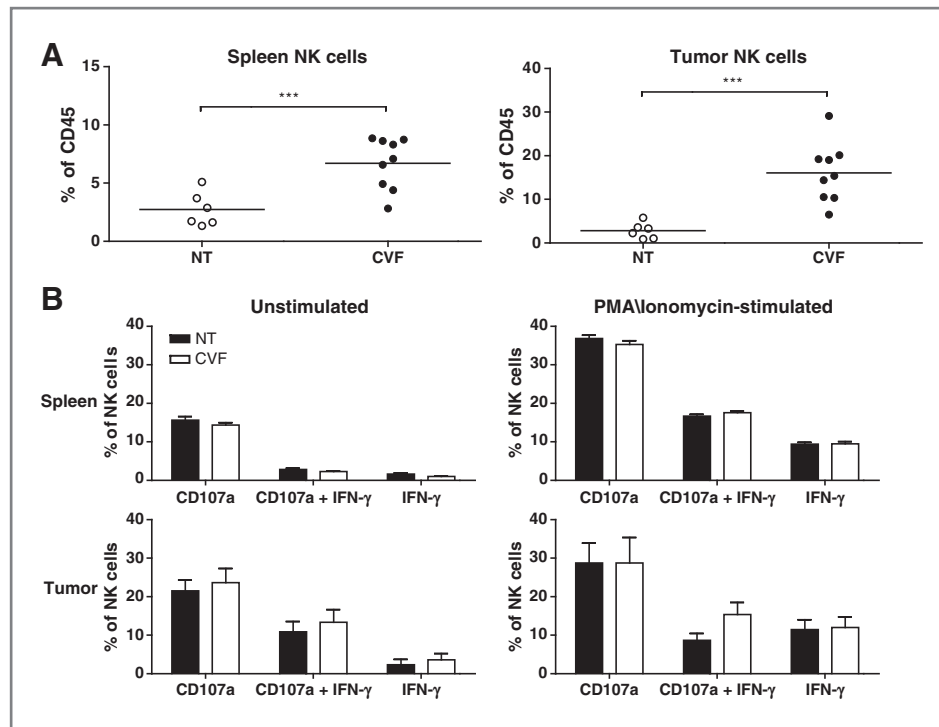


Figure 2. Decomplementation increases NK cell availability without affecting their effector function. B16gp33-bearing mice were treated or not with CVF. On day 9 following treatment, spleens and tumors were harvested. A, percentage of NK cells among CD45⁺ cells. Mean \pm SEM are shown ($n = 9$). ***, $P < 0.001$. B, NK degranulation assay in YAC-1 cocultures (10:1 effector–target ratio) with or without PMA–ionomycin. Data are the mean \pm SEM of three independent experiments ($n = 9$). NT, not treated.

NK intracellular staining assay

For IFN- γ intracellular staining, single-cell suspensions were prepared from the spleens and tumors harvested 9 days after CVF injection. Cells were cocultured with YAC-1 cells (provided by Dr. Suzanne Lemieux; at 10:1 effector–target ratio) with or without phorbol 12-myristate 13-acetate (PMA)–ionomycin. Cytokine production was measured in the presence of monensin A (20 $\mu\text{g}/\text{mL}$), brefeldin A (10 $\mu\text{g}/\text{mL}$), and anti-CD107a FITC antibody for 5 hours. Cells were stained for surface markers, fixed, and permeabilized for intracellular staining using fixation and permeabilization buffers from BioLegend according to the manufacturer's instructions. IFN- γ -PE and CD107a-FITC were obtained from BioLegend.

T lymphocyte intracellular staining assay

For IFN- γ and TNF- α intracellular staining, single-cell suspensions were prepared from the spleens (3 mice/group) 9 days after CVF injection. Cytokine production in response to the tumor antigen was measured by incubation with the gp33 peptide (KAVYNFATC 1 $\mu\text{g}/\text{mL}$) in the presence of brefeldin A (10 $\mu\text{g}/\text{mL}$) and interleukin (IL)-2 (100 U/mL) for 5 hours. Cells were stained for surface markers then fixed and permeabilized for intracellular staining using fixation and permeabilization buffers as described above. For degranulation assay and granzyme B intracellular staining, cells were incubated with the peptides in the presence of monensin A (20 $\mu\text{g}/\text{mL}$) and anti-CD107a FITC antibody for 5 hours. Cells were stained for surface markers, then fixed and permeabilized for intracellular staining. IFN- γ -PE and TNF- α -APC were obtained from BioLegend and granzyme B eFluor 450 was purchased from eBiosciences.

Statistical analyses

A Student t test was used for group comparison, and P values of less than 0.05 were considered statistically significant.

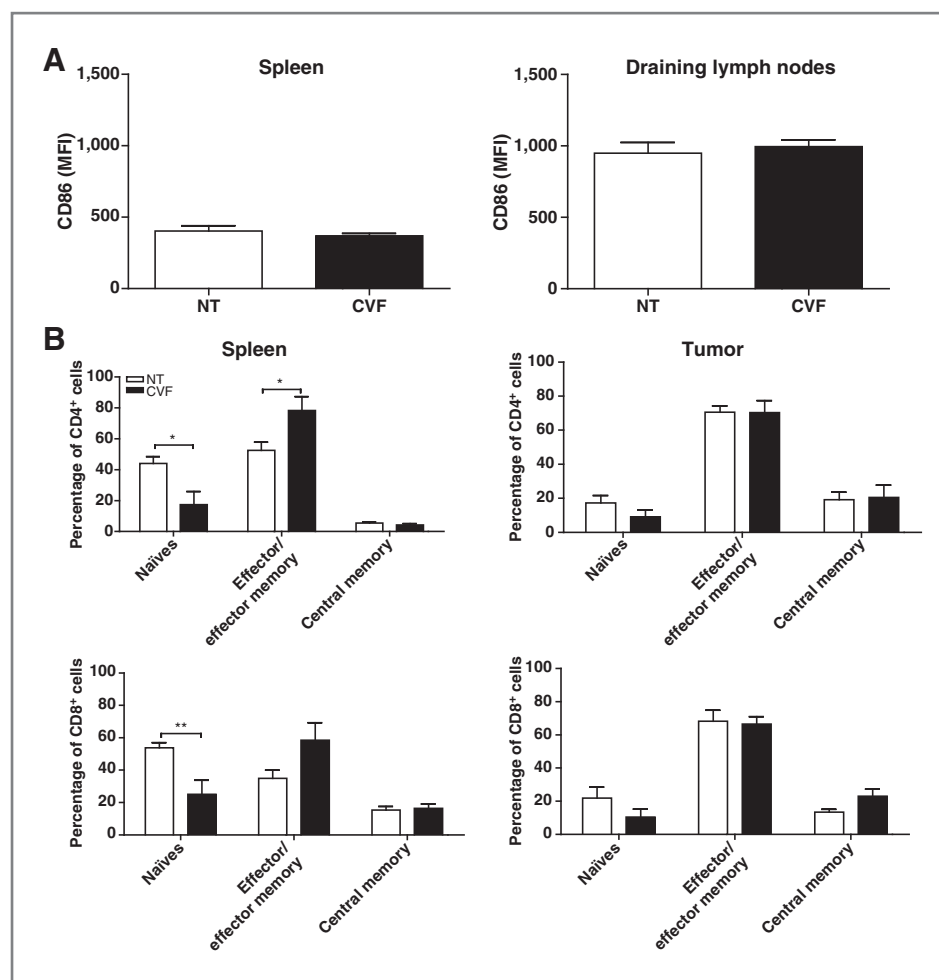
Results and Discussion

CVF-mediated decomplementation slows down early tumor progression and promotes immune cell infiltration

In recent years, there has been renewed interest in the role of complement in various disease conditions. In cancer, the role of complement is unclear since it has been reported to play a role in both tumor clearance and progression (2, 3). Nevertheless, studies have indicated that blocking complement proteins might improve the efficacy of antitumor immunotherapy (16). Some complement activation products, such as the anaphylatoxins C3a and C5a, are potent proinflammatory mediators, and it is now well recognized that inflammation is able to both promote and exacerbate tumor growth.

To establish the role of the complement system in the generation of an antitumoral immune response, transient decomplementation was performed using CVF 6 days following tumor administration, when T-cell priming is expected to occur. CVF is a structural and functional analogue of the C3 molecule, which acts as a C3/C5 convertase. CVF-containing convertases are more stable and resistant to regulatory inhibitors and deplete complement activity by C3 consumption (17). Unlike the complement protein knockout mice (e.g., C3^{-/-}, C4^{-/-}, or C5^{-/-}), CVF does not affect the splenic architecture and thus allows a complete and unaltered immune response to be mounted and analyzed (18). Because

Figure 3. Increased systemic T-cell activation following CVF treatment. A, spleens and draining lymph nodes were harvested 9 days following CVF treatment, and dendritic cell activation was assessed by CD11c and CD86 staining. Data are the mean \pm SEM of two independent experiments ($n = 6$). B, T-cell activation was assessed by CD62L and CD44 staining on spleens and tumors. Naïve ($CD62L^+CD44^-$), effector/effector memory ($CD62L^-$), and central memory T cells ($CD62L^+CD44^+$) are shown. Data are the mean \pm SEM of three independent experiments ($n = 9$). *, $P < 0.05$; **, $P < 0.01$. NT, not treated.



CVF-mediated complement depletion lasts approximately 3 days in treated mice (Supplementary Fig. S1), we hypothesized that this short period of time could affect T-cell priming. CVF-mediated transitory decomplementation soon after tumor implantation efficiently led to a significant slowdown of the growth of B16 melanoma tumors (Fig. 1A). CVF treatment induced a significant increase in the percentage of tumor-infiltrating immune cells 9 days following decomplementation (Fig. 1B). Strikingly, CVF treatment modulated the composition of the infiltrating immune cells; it affected the NK cell proportions, but had little to no effect on the proportions of $CD4^+$ T cells, regulatory T cells (Treg), $CD8^+$ T cells, B cells, neutrophils, macrophages, and dendritic cells (Fig. 1C).

An important mechanism used by cancer cells to suppress immune responses against tumor antigens is abnormal myelopoiesis and the recruitment of myelomonocytic cells to the tumor microenvironment and peripheral lymphoid organs (19). In concordance with a study conducted by Markiewski and colleagues (5), complement inhibition also significantly decreased the frequency of myeloid-derived suppressor cells (MDSC) in the tumor and the spleen (Fig. 1C and Supplementary Fig. S2). MDSC are a population of intermediately differentiated myeloid cells known to inhibit cancer

immunosurveillance and therefore potentiating neoplastic proliferation (2). These cells express high levels of membrane $C5aR$ (5). $C5a/C5aR$ binding on MDSC promotes their migration and increases their production of immunosuppressive molecules such as reactive oxygen and nitrogen species, consequently inhibiting cytotoxic $CD8^+$ T lymphocytes and NK cells and stimulating tumorigenic cytokine production and angiogenesis (5, 20). MDSC can also be recruited to tumors by the anaphylatoxin $C5a$ deposited in the tumor vasculature (5).

CVF treatment promotes a better NK cell availability

NK cell proportions were greatly increased following decomplementation both in the tumor (8-fold) and in the spleen (3-fold; Fig. 2A). We next assessed whether decomplementation also modulated NK cell function. Tumoral and splenic NK cells harvested 9 days after CVF treatment were not impaired functionally compared with nontreated animals, as determined by degranulation assay ($CD107a$) and $IFN-\gamma$ production after exposure to YAC-1 target cells. Furthermore, NK cells retained their ability to respond to *ex vivo* stimulation (Fig. 2B). Complement proteins are known to induce the production of $TGF-\beta$ (21), which may facilitate angiogenesis, invasion, and metastasis (22), and downregulate the expression of $IL-2R\beta$ as

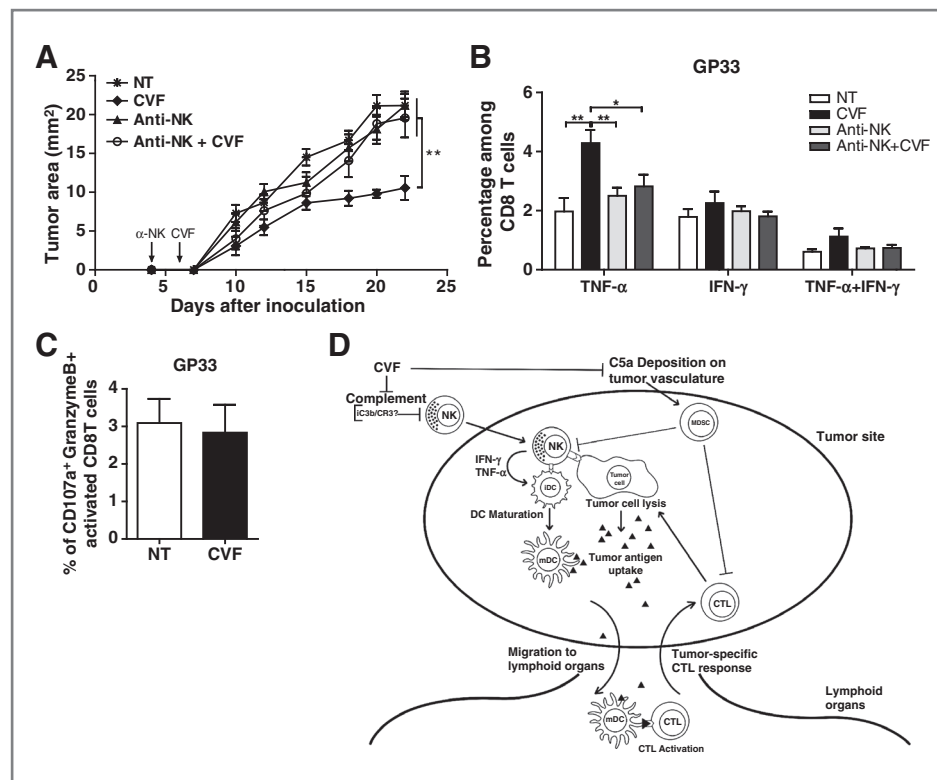


Figure 4. Influence of NK cells on CVF therapy. **A**, C57BL/6 mice were injected s.c. with B16 cells ($n = 6-8$), NK depleted or not at day 4 and treated or not with CVF at day 6. Tumor growth was measured at indicated time points. Data show the mean \pm SEM and are representative of two independent experiments. *, $P < 0.05$; **, $P < 0.01$. **B**, C57BL/6 mice were injected s.c. with B16gp33 cells, NK depleted or not at day 4 and treated or not with CVF at day 6. Nine days following CVF treatment, spleens were harvested and an *ex vivo* stimulation with the gp33 (KAVYNFATC) peptide was performed to analyze cytokine secretion and C, degranulation. Data are the mean \pm SEM of two independent experiments ($n = 6$). **D**, the proposed model for the influence of complement proteins and NK cells in the B16 melanoma model. Inhibition of the complement system by CVF allows for increased NK cell availability in tumor tissues. This expanded proportion of NK cells leads to increased tumor cell lysis and antigen release, thus facilitating the activation of tumor-specific CD8⁺ T lymphocytes. Inhibition of complement proteins also acts on MDSC and limits the immunosuppressive tumor microenvironment, leading to increased CTL activation. iDC, immature dendritic cell; mDC, mature dendritic cell; NT, not treated.

well as the secretion of IFN- γ by NK cells (23). Our results suggest that it is unlikely that this pathway is involved here because NK cells harvested from CVF-treated mice are fully functional and respond normally.

The complement system limits systemic T-cell activation

It is well recognized that the clearance of apoptotic cells through iC3b opsonization and CR3 phagocytosis can be accompanied by a reduction in the expression of costimulatory molecules and an impaired maturation of dendritic cells (24). The recruitment of large numbers of NK cells may therefore be critical for optimal dendritic cell activation and the subsequent induction of T-cell responses in these low-inflammation conditions. Given that NK cells can cross talk with dendritic cells to mediate their activation, we assessed the activation status of dendritic cells after transient inhibition of the complement system. We did not observe any modulation of the CD86 expression levels in splenic or draining lymph node dendritic cells (Fig. 3A). We then determined whether the enhanced NK cell proportions at the tumor site could lead to an increase in T-cell activation. The majority of CD4⁺ and CD8⁺ T cells present in the tumor were of the effector/effector-memory phenotype

and their proportions did not change following complement inhibition. However, in the spleen, CVF-mediated depletion of complement led to a reduction in the proportions of naïve T cells associated with increased proportions of effector CD4⁺ and CD8⁺ T cells (Fig. 3B). It is therefore likely that, in the absence of complement proteins, functional dendritic cells have access to more tumor antigens due to increased NK-mediated cell lysis allowing for a better antigen presentation to the CD8⁺ T lymphocytes.

The enhancement of tumor-specific CTL responses in decompemented mice is NK dependent

It has been reported that the complement components can interfere with the binding of NK cells to monoclonal antibodies (mAb) used in certain cancer treatments thus limiting NK cell-mediated lysis of antibody-coated target cells (25). This inhibition was shown to be dependent on C3b, likely by its binding to CR3 molecules abrogating signaling via Fc γ receptor III (CD16; ref. 25). Furthermore, the depletion of complement has been shown to enhance the survival rates following mAb therapy in a syngeneic mouse model of lymphoma (26).

To ascertain whether the enhanced tumor control in the decomplemented mice was a direct consequence of the increased proportions of NK cells, we depleted NK cells 4 days after tumor implantation. It is important to note that *in vivo* depletion with anti-NK1.1 antibody is complement mediated, thus required before CVF treatment. We found that in the absence of NK cells, CVF treatment had no effect on tumor implantation and early tumor development, demonstrating that NK cells were necessary for the decreased tumor growth observed following decomplementation (Fig. 4A).

Considering that T-cell activation in the spleen is also increased in the absence of complement, we determined the effect of decomplementation on the induction of tumor-specific CTLs. As assessed by intracellular cytokine staining of gp33 peptide-stimulated T cells from decomplemented and control B16gp33-bearing mice, we found that complement inhibition led to higher levels of tumor-specific (gp33) T cells secreting mainly TNF- α (Fig. 4B). Accordingly, the enhancement of gp33-specific CTL induction by CVF was also abolished in the absence NK cells, indicating that the effect of decomplementation on early tumor growth is largely mediated by CTLs (Fig. 4B). However, CVF-mediated decomplementation did not lead to superior levels of gp33-specific cytotoxicity as assessed by CD107a expression, a marker of recent degranulation (Fig. 4C). Thus, using the B16 melanoma model, we demonstrate that CVF treatment limits early tumor growth and promotes enhanced availability of the splenic and tumoral NK cells.

Therefore, our study has identified a previously unrecognized function for complement in tumor biology. We have shown that complement components and NK cells interact and influence tumor growth by limiting tumor-specific CTL induction. Factors from the complement system may restrict NK cell availability in tumor tissue in which NK cells would normally participate in the induction of a tumor-specific CTL response via, among other things, tumor cell lysis. Furthermore, complement proteins could also promote infiltration of the tumor by MDSCs that could then suppress lymphocyte function (Fig. 4D).

Many drugs targeting complement proteins are currently in clinical trials, and few side effects have been reported (1, 27).

References

- Ricklin D, Lambris JD. Complement-targeted therapeutics. *Nat Biotechnol* 2007;25:1265–75.
- Ostrand-Rosenberg S. Cancer and complement. *Nat Biotechnol* 2008;26:1348–9.
- Gelderman KA, Tomlinson S, Ross GD, Gorter A. Complement function in mAb-mediated cancer immunotherapy. *Trends Immunol* 2004;25:158–64.
- Venkatesha RT, Thangam EB, Zaidi AK, Ali H. Distinct regulation of C3a-induced MCP-1/CCL2 and RANTES/CCL5 production in human mast cells by extracellular signal regulated kinase and PI3 kinase. *Mol Immunol* 2005;42:581–7.
- Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. *Nat Immunol* 2008;9:1225–35.
- Rutkowski MJ, Sughrue ME, Kane AJ, Mills SA, Parsa AT. Cancer and the complement cascade. *Mol Cancer Res* 2010;8:1453–65.
- Moretta A, Bottino C, Mingari MC, Biassoni R, Moretta L. What is a natural killer cell? *Nat Immunol* 2002;3:6–8.
- Arase N, Arase H, Park SY, Ohno H, Ra C, Saito T. Association with Fc γ R1 is essential for activation signal through NKR-P1 (CD161) in natural killer (NK) cells and NK1.1+ T cells. *J Exp Med* 1997;186:1957–63.
- Roda JM, Parihar R, Lehman A, Mani A, Tridandapani S, Carson WE. Interleukin-21 enhances NK cell activation in response to antibody-coated targets. *J Immunol* 2006;177:120–9.
- Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter JM, et al. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate antitumor immune responses *in vivo*. *Nat Med* 1999;5:405–11.
- Vitale M, Chiesa MD, Carlomagno S, Pende D, Aricò M, Moretta L, et al. NK-dependent DC maturation is mediated by TNF- α and IFN- γ released upon engagement of the Nkp30 triggering receptor. *Blood* 2005;106:566–71.

CVF has been used in laboratory animals for the treatment of various diseases. One of the limits of this treatment alternative in the clinic is the high immunogenicity of this compound (28). However, a human C3 hybrid, containing crucial regions of the CVF β chain (so-called humanized CVF or HC3-1496), has been created for this purpose (29). It will be of great interest to test other complement inhibitors currently in development in various solid tumor models to determine whether sustained treatment will favor the induction of an improved tumor-specific immune response and promote CD8⁺ T-cell expansion. Thus, a better understanding of the link between the complement proteins and NK cells in the developing tumors could lead to the design of new therapeutic strategies against cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: V. Janelle, A. Lamarre

Development of methodology: V. Janelle, M.-P. Langlois, E. Tarrab, A. Lamarre

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Janelle, M.-P. Langlois

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): V. Janelle, M.-P. Langlois, P. Lapierre, A. Lamarre

Writing, review, and/or revision of the manuscript: V. Janelle, P. Lapierre, L. Poliquin, A. Lamarre

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V. Janelle, M.-P. Langlois

Study supervision: V. Janelle, E. Tarrab, L. Poliquin, A. Lamarre

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12. Piccioli D, Sbrana S, Melandri E, Valiante NM. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J Exp Med* 2002;195:335–41.
13. Caron E, Hall A. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* 1998;282:1717–21.
14. Muto S, Veřtvička V, Ross G. CR3 (CD11b/CD18) expressed by cytotoxic T cells and natural killer cells is upregulated in a manner similar to neutrophil CR3 following stimulation with various activating agents. *J Clin Immunol* 1993;13:175–84.
15. Prevost-Blondel A, Zimmermann C, Stemmer C, Kulmburg P, Rosenthal FM, Pircher H. Tumor-infiltrating lymphocytes exhibiting high *ex vivo* cytolytic activity fail to prevent murine melanoma tumor growth *in vivo*. *J Immunol* 1998;161:2187–94.
16. Macor P, Tedesco F. Complement as effector system in cancer immunotherapy. *Immunol Lett* 2007;111:6–13.
17. Vogel CW, Smith CA, Müller-Eberhard HJ. Cobra venom factor: structural homology with the third component of human complement. *J Immunol* 1984;133:3235–41.
18. Marsh JE, Farmer CK, Jurcevic S, Wang Y, Carroll MC, Sacks SH. The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. *Transplantation* 2001;72:1310–8.
19. Gallina G, Dolcetti L, Serafini P, Santo CD, Marigo I, Colombo MP, et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8⁺ T cells. *J Clin Invest* 2006;116:2777–90.
20. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436–44.
21. Gionanlis L, Alexopoulos E, Papagianni A, Leontsini M, Memmos D. Fibrotic mechanisms in idiopathic rapidly progressive glomerulonephritis: the role of TGF- β 1 and C5b-9. *Ren Fail* 2008;30:239–46.
22. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet* 2001;29:117–29.
23. Penafuerte C, Galipeau J. TGF β secreted by B16 melanoma antagonizes cancer gene immunotherapy bystander effect. *Cancer Immunol Immunother* 2008;57:1197–206.
24. Morelli AE, Larregina AT, Shufesky WJ, Zahorchak AF, Logar AJ, Papworth GD, et al. Internalization of circulating apoptotic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. *Blood* 2003;101:611–20.
25. Wang S-Y, Racila E, Taylor RP, Weiner GJ. NK-cell activation and antibody-dependent cellular cytotoxicity induced by rituximab-coated target cells is inhibited by the C3b component of complement. *Blood* 2008;111:1456–63.
26. Wang S-Y, Veeramani S, Racila E, Cagley J, Fritzing DC, Vogel C-W, et al. Depletion of the C3 component of complement enhances the ability of rituximab-coated target cells to activate human NK cells and improves the efficacy of monoclonal antibody therapy in an *in vivo* model. *Blood* 2009;114:5322–30.
27. Kohl J. Drug evaluation: the C5a receptor antagonist PMX-53. *Curr Opin Mol Ther* 2006;8:529–38.
28. Gowda DC, Petrella EC, Raj TT, Bredehorst R, Vogel CW. Immunoreactivity and function of oligosaccharides in cobra venom factor. *J Immunol* 1994;152:2977–86.
29. Fritzing DC, Hew BE, Thorne M, Pangburn MK, Janssen BJC, Gros P, et al. Functional characterization of human C3/cobra venom factor hybrid proteins for therapeutic complement depletion. *Dev Comp Immunol* 2009;33:105–16.

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