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Antitumor IgE Adjuvanticity: Key Role of FcεRI[1]

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Working with C57BL/6 mouse tumor models, we had previously demonstrated that vaccination with IgE-coated tumor cells can protect against tumor challenge, an observation that supports the involvement of IgE in antitumor immunity. The adjuvant effect of IgE was shown to result from eosinophil-dependent priming of the T cell-mediated adaptive immune response. The protective effect is likely to be mediated by the interaction of tumor cell-bound IgE with receptors, which then trigger the release of mediators, recruitment of effector cells, cell killing and tumor Ag cross-priming. It was therefore of utmost importance to demonstrate the strict dependence of the protective effect on IgE receptor activation. First, the protective effect of IgE was confirmed in a BALB/c tumor model, in which IgE-loaded modified VV Ankara-infected tumor cells proved to be an effective cellular vaccine. However, the protective effect was lost in FcεRIα$^{-/-}$ (but not in CD23$^{-/-}$) knockout mice, showing the IgE-FcεRI interaction to be essential. Moreover, human IgE (not effective in BALB/c mice) had a protective effect in the humanized knockin mouse (FcεRIα$^{-/-}$ hFcεRIα^{+}). This finding suggests that the adjuvant effect of IgE could be exploited for human therapeutics. ***The Journal of Immunology,*** **2009, 183: 4530–4536.**

Human IgE is a powerful effector in anti-parasitic immunity and plays a central role in allergic manifestations (1). In addition to these well-known functions, new physiological roles, not clearly defined yet, have been proposed during the last decades. A possible involvement of IgE in many different disorders like diabetes mellitus, cardiovascular diseases, and tumors has been suggested (2). With respect to tumors, controversial epidemiological studies propose the allergic condition either as a risk or a protective factor, depending on the kind of tumor and allergy (3, 4). Overall, these data often show an inverse correlation between the two conditions, especially for pancreatic cancer (5–7), childhood leukemia (8, 9), brain cancers (10–12), and ovarian cancer (13). Starting from these lines of evidence, it has been proposed to exploit IgE effector potency in the immunotherapy of cancer. All the studies performed to investigate a possible use of IgE in antitumor therapy highlight the efficacy of IgE targeting against tumor Ags to generate an antitumor effect. The first use of an IgE specific for a tumor-associated Ag (TAA)[3] was performed in 1991 by Nagy et al. (14), who developed a mouse IgE mAb against a mouse mammary tumor virus and showed that it prevented tumor development. A similar result was obtained by Kershaw et al. (15) in 1998 with a TAA-specific IgE mAb in a colorectal adenocarcinoma model in SCID mice. In 1999, the Gould group (16) generated a chimeric IgE mAb derived from the original MOv18 IgG, a mAb specific for an ovarian carcinoma TAA (16), the folate binding protein (17). A better and long lasting antitumor effect of MOv18 IgE vs MOv18 IgG was observed in a xenograft model of ovarian carcinoma (18), followed by the observation that human monocytes were the main cell type involved in the antitumor response (19). More recently, it has been proposed that monocytes exert the IgE-dependent killing of ovarian tumor cells in vitro by both FcεRI-dependent Ab-dependent cellular cytotoxicity and CD23-dependent Ab-dependent cellular phagocytosis (20, 21). A different way to study the potential relevance of IgE Abs in tumor protection was elaborated by the Jensen-Jarolim group (22, 23) that developed an oral vaccination protocol with tumor Ags in mice under antiacid medication. Oral immunization of mice with mimotopes of trastuzumab, an anti-human epidermal growth factor receptor (HER)-2 mAb, led to the development of HER-2-specific IgE. HER-2-specific IgE-bound tumor cells could be killed in vitro by FcεRI^{+} effector cells (24).

Our first approach in the study of an IgE-mediated immune response in the prevention and control of tumor growth relied on the in vitro or in vivo targeting of IgE on the surface of tumor cells by a three-step strategy (25). The strategy consisted in the creation of an avidin bridge between a biotinylated TAA-specific Ab and an unspecific IgE mAb, to cover tumor cells with IgE. In that study IgE strongly influenced both tumor size and growth rate, leading to tumor-specific acquired immunity through CD4^{+} and CD8^{+} T cells priming. Interestingly, immunization of mice with irradiated IgE-loaded tumor cells conferred protection against subsequent challenges with untreated tumor cells. This effect has been investigated and observed in two tumor models: the highly immunogenic RMA lymphoma T cell line and the weakly immunogenic MC38 colon adenocarcinoma cell line (25). Consequently, we decided to study the adjuvant role of IgE in a cell-based antitumor vaccine investigating the relevance of IgE interaction with its receptors. To this aim, we exploited the availability of mouse strains knocked out for FcεRIα or CD23 (26, 27). Furthermore, by applying a vaccination protocol using human IgE to a human FcεRIα transgenic mouse (28), we progressed toward a humanized system,

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[3] Abbreviations used in this paper: TAA, tumor-associated Ag; DNFB, 2,4-dinitro-1-fluorobenzene; HER-2, human epidermal growth factor receptor 2; HSA, human serum albumin; VV, vaccinia virus; MVA, modified VV Ankara; WT, wild type.

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generating a suitable background for the translation of these results into possible clinical applications.

Materials and Methods

Mice

Experiments conducted on mice were performed in accordance with institutional and state guidelines. C57BL/6 mice (females 8 wk of age) were purchased from Charles River Laboratories; BALB/c mice (female 8 wk of age) were purchased from Harlan Laboratories. FcεRIα$^{-/-}$ and FcεRIα$^{-/-}$ hFcεRIα^{+} (BALB/c background) mice were previously described (26, 28). CD23$^{-/-}$ (BALB/c background) mice were provided by Dr. P. Yu (University of Marburg, Marburg, Germany) (27). Transgenic mice were bred in conventional animal facility.

Cell lines

RMA is a Rauscher MuLV-induced H-2^{b} T cell lymphoma of C57BL/6 origin, maintained in RPMI 1640 medium (Life Technologies). TS/A-LACK (*Leishmania* receptor for activated C kinase) is a H-2^{d} mammary adenocarcinoma of BALB/c origin, provided by Dr. A. Mondino (San Raffaele Scientific Institute, Milan, Italy), maintained in RPMI 1640 medium supplemented with 100 μg/ml geneticin (G418; Calbiochem). RBL-2H3 is a rat basophilic cell line maintained in DMEM (Life Technologies). RBL-SX38 is a RBL-2H3-derived cell line expressing the α-, β-, and γ-chain of human FcεRI (29), maintained in DMEM and 800 μg/ml G418. All cultures were supplemented with 100 U/ml penicillin/streptomycin (Life Technologies) and 10% FCS (Euroclone).

Virus

Vaccinia virus (VV) is a VV Weith (Lederle). MKG is a modified VV Ankara (MVA) with K1L in deletion III and enhanced GFP in deletion VI, a gift of Prof. V. Erfle (Munich Technical University, Munich, Germany). Virus stocks were prepared on BHK-21 cells by conventional techniques.

IgE loading of tumor cells by haptenization

Mouse IgE loading was conducted incubating tumor cells with 2.5 mM DNFB (2,4-dinitro-1-fluorobenzene; Sigma-Aldrich) in PBS (10^{6} cells/ml) for 20 min at room temperature. Following a 10 min blocking reaction with RPMI 1640 medium supplemented with 10% FCS and washing, cells were incubated with 20 μg/ml anti-DNP mouse IgE mAb (SPE7; Sigma-Aldrich) in PBS containing 5% BSA for 45 min at 4°C. Human IgE loading was conducted incubating tumor cells with NIP-OSu (4-hydroxy-5-iodo-3-nitrophenylacetyl-hydroxysuccinimidyl ester; Cambridge Research Biochemicals), 0.2 mg/10^{6} cells in 10 mM sodium phosphate (pH 7.2) (10^{6} cells/ml) for 45 min at room temperature. Following a 10 min blocking reaction with RPMI 1640 medium supplemented with 10% FCS and washing, cells were incubated 45 min at 4°C in PBS containing 5% BSA with 20 μg/ml purified chimeric anti-NIP (4-hydroxy-5-iodo-3-nitrophenylacetyl) human IgE mAb secreted by a stably transfected J558L cell clone, as previously reported (30).

Cytofluorimetry

IgE loading was monitored by cytofluorimetry incubating 5×10^{6} tumor cells with 5 μg/ml biotinylated rat anti-mouse IgE mAb (BD Pharmingen) or biotinylated mouse anti-human IgE mAb (BD Pharmingen), in PBS containing 5% BSA, for 30 min at 4°C. After washing, cells were incubated with PE-conjugated streptavidin (BD Pharmingen) in PBS containing 5% BSA for 20 min at 4°C and fixed with PBS containing 2% formaldehyde.

Mediator release

Plastic-adherent RBL-2H3 (or RBL-SX38 for human IgE) cells (8×10^{4} cells/well) were incubated in DMEM with 100 ng mouse anti-DNP IgE mAb (or anti-NIP human IgE mAb) for 2 h at 37°C. Cells were then washed and incubated in triggering buffer (119 mM NaCl, 5 mM KCl, 25 mM PIPES, 5.6 mM glucose, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 0.1% BSA (pH 7.2)) with 100 ng human serum albumin (HSA)-DNP (Sigma-Aldrich) or NIP-BSA (Biosearch Technologies) for 1 h at 37°C. Alternatively, IgE-loaded or not loaded tumor cells in triggering buffer were added to RBL-2H3 (or RBL-SX38 for human IgE) cells (at a 2:1 ratio). Plates were centrifuged 5 min at 300 × *g* and incubated for 1 h at 37°C. The release of β-hexosaminidase by RBL-2H3 (or RBL-SX38 for human IgE) was detected in the cell supernatant. Supernatants were transferred to a new plate and 1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosamide (Sigma-Aldrich) in 0.1 M citrate buffer (pH 6.2) was added, followed by a 150-min incubation at 37°C. The reaction was stopped using 0.1 M stop solution (0.1 M Na_2CO_3, 0.1 M $NaHCO_3$, (pH 10.0)), and the absorbance was read at 405 nm. Negative control was the supernatant of nonstimulated cells. Positive control was the supernatant of IgE-sensitized RBL-2H3 cells stimulated with 100 ng HSA-DNP (or human IgE-sensitized RBL-SX38 cells stimulated with 100 ng NIP-BSA). The positive control represented 70% of the total β-hexosaminidase content obtained from cell lysis with 0.1% Triton X-100. The results are calculated as percentage of total β-hexosaminidase content using $100 \times (A_{405}$ sample $\times A_{405}$ negative control)/(A_{405} β-hexosaminidase content $\times A_{405}$ negative control).

Vaccination experiments

Immunizations were performed on five mice for each group by s.c. administration of VV or MVA-infected IgE-loaded tumor cells (RMA cells for C57BL/6 mice and TS/A-LACK cells for BALB/c mice) (10^{5} cells/mouse) in PBS at the base of the tail. VV or MVA-infected tumor cells were used as a control. Immunization was performed once or twice at a 2-wk interval. Two weeks after the last immunization, mice were challenged by s.c. injection in the left flank with 7×10^{4} RMA cells (C57BL/6) and 2×10^{5} TS/A-LACK cells (BALB/c) in PBS. Tumor growth was monitored at 1–3 day intervals by measuring tumor volume with a caliper. Tumor volume, *V*, was calculated by the equation $4/3\pi \times r_1 \times r_2 \times r_3$, where r_1 is the longitudinal radius, r_2 is the lateral radius, and r_3 is the thickness of tumors protruding from the surface of normal skin (31).

Statistics

In each set of experiments we assume that the tumor growth follows an exponential pattern as function of time. More specifically, we assume that $v_{ijt} = \alpha_i e^{\beta_i t} \varepsilon_{ijt}$, where v_{ijt} is the tumor volume measured in the *i*-th experimental line, in the *j*-th mice at time *t*. The parameters β_i are fixed-effect coefficients that give the measure of tumor volume progression; α_i are random-effect coefficients for group *i* and they are assumed to be independent log normally distributed. These parameters are thought as random variables and describe unwilled possibly random differences in initial conditions at $t = 0$ between groups; ε_{ijt} is the observation error for measurement made at *t* on *j*-th mice of the *i*-th experimental line. Errors ε_{ijt} are assumed to have a multivariate log-normal distribution. Mixed random fixed-effect models (32) are successfully applied in a number of studies (33–37). Because observations represent longitudinal data on single individuals, we considered a first order autocorrelation structure among errors. Furthermore, errors in our model are assumed to have multiplicative effect on observed measurements. Such assumptions guarantee that observed measurements are always positive with variance monotonically increasing with the tumor volume, which is empirically observed. The hypothesis tested concerned differences between progressions (H_0:$\beta_i = \beta_{i'}$, H_1:$\beta_i \neq \beta_{i'}$, $\forall i \neq i'$). It can be shown that these testing procedures lead to *t*-type tests (32). Statistical significance was defined as a value of $p < 0.05$.

Results

Tumor cells loaded with anti-hapten IgE trigger mediator release

The IgE system is governed by interactions between IgE and its receptors (FcεRI and CD23), which regulate both IgE production and very strong immune responses (1). In a previous study, we demonstrated that protective immunity can be elicited by vaccination of mice with IgE-coated tumor cells (25). In the present study, tumor cells were haptenized with DNP and loaded with a DNP-specific IgE mAb (38). This approach can be exploited for many different tumors, as it does not depend on specific TAA expression. IgE loading was validated through cytofluorimetric analysis, detecting the presence of IgE on the surface of tumor cells by a biotinylated anti-mouse IgE mAb followed by streptavidin-PE (Fig. 1*A*). In addition, the capacity of cell surface-bound IgE to bind and activate FcεRI was monitored by a mediator release assay (Fig. 1*B*). When IgE-loaded tumor cells were incubated with the rat basophilic cell line RBL-2H3, expressing FcεRI, degranulation and mediator release were triggered via FcεRI cross-linking, as previously reported for membrane IgE (30). IgE on tumor cells induced a β-hexosaminidase release >60% of the maximum release obtained upon cell lysis with Triton X-100. This demonstrates a powerful FcεRI cross-linking, as the β-hexosaminidase release is similar to that obtained by cross-linked soluble IgE

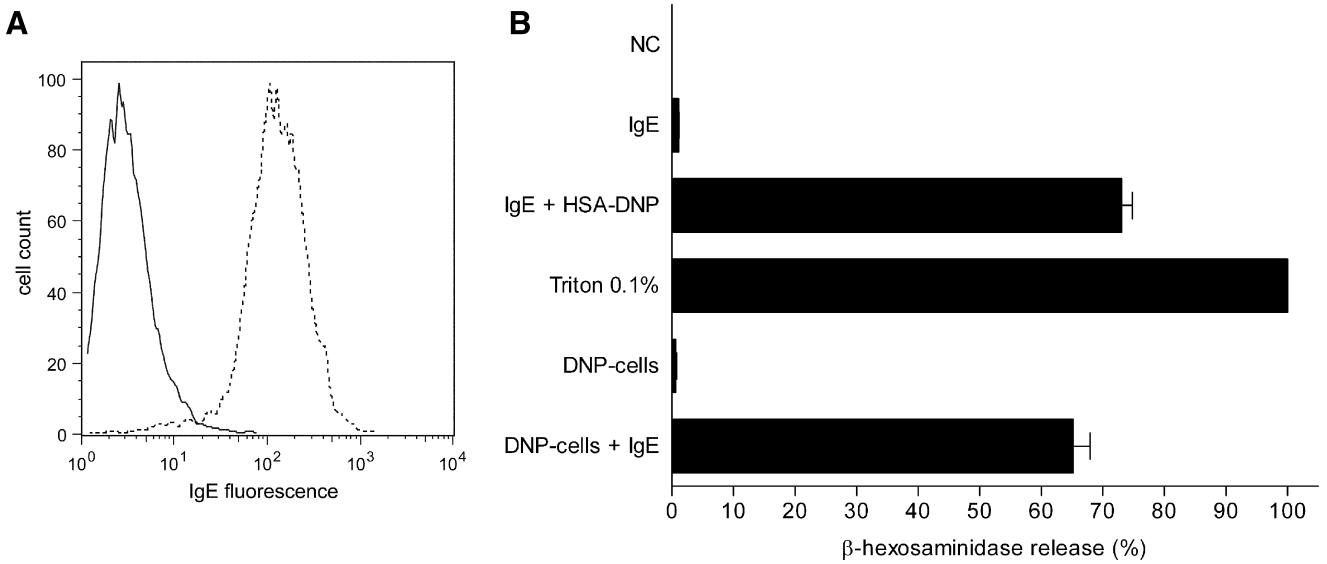

FIGURE 1. IgE loading onto tumor cells triggers FcεRI$^+$ cells. *A*, Cell surface loading of IgE. Tumor cells were haptenized with DNFB and loaded with mouse anti-DNP IgE (dotted histogram) or not loaded (thin line histogram). IgE loading was verified by cytofluorimetric analysis using biotinylated anti-mouse IgE followed by streptavidin-PE. *B*, IgE-mediated β-hexosaminidase release assay. RBL-2H3 cells were put in contact with IgE-loaded (DNP-cells + IgE) or not loaded (DNP-cells) haptenized tumor cells. A 100% release corresponds to the release obtained by lysing cells with 0.1% Triton X-100. Positive control consists of anti-DNP soluble IgE-sensitized RBL-2H3 cells cross-linked with HSA-DNP (IgE + HSA-DNP). Negative controls (NC): nonstimulated RBL-2H3 cells; anti-DNP soluble IgE-loaded RBL-2H3 cells in the absence of HSA-DNP (IgE). Results are mean ± SD of three determinations.

(~70%). In contrast, tumor cells not loaded with IgE or loaded with IgG (data not shown) did not induce mediator release.

IgE-loaded MVA-infected tumor cells are efficient cellular vaccines

We introduced VV and MVA into our tumor cell immunization protocol for several reasons: 1) to avoid the need to kill tumor cells by irradiation (vaccinia infection kills the cells in a few days); 2) to exploit the high immunogenicity of vaccinia; and 3) to establish a system (recombinant MVA) suitable to induce cell surface IgE expression. After encouraging preliminary experiments with VV (data not shown) we switched to MVA, a highly attenuated form of VV with a severe host restriction (39). The lack of pathogenicity for mammals, the high-level expression of foreign Ags and the adjuvant effect on immune responses make MVA an ideal vector for both prophylactic and therapeutic vaccination (40). We therefore studied the IgE adjuvant effect in MVA-infected tumor cells. C57BL/6 mice received either a single immunization or two immunizations 15 days apart, comprising 10^5 IgE-loaded MVA-infected RMA cells. IgE-free MVA-infected RMA cells were used as a control. Immunization with IgE-loaded MVA-infected RMA cells elicited protection after a single immunization, whereas IgE-free MVA-infected RMA cells induced protection only after two immunizations (Fig. 2). The different tumor growth in mice receiving one immunization with or without IgE was highly significant ($p = 0.0006$). Therefore, IgE also exerts its adjuvant effect with cellular vaccines based on MVA-infected tumor cells.

Confirmation of IgE antitumor adjuvanticity in a BALB/c tumor model

The IgE adjuvant effect could depend on the interaction with FcεRI or CD23. Hence, IgE receptor involvement was investigated using FcεRIα$^{-/-}$ and CD23$^{-/-}$ mice (26, 27). As both knockout mice were generated in a BALB/c background, it was first necessary to reproduce in BALB/c mice the results obtained using C57BL/6 mice. To this aim, we used the BALB/c mammary adenocarcinoma cell line TS/A-LACK (41). As with the C57BL/6 model, BALB/c mice were vaccinated either once or twice (15 days apart) with IgE-loaded MVA-infected TS/A-LACK cells and challenged 15 days after the last immunization. IgE-free MVA-infected TS/A-LACK cells were used as a control. The degree of tumor protection in this BALB/c model was very similar to that reported for the C57BL/6 model. Indeed, the IgE adjuvant effect was achieved after a single immunization with IgE-loaded

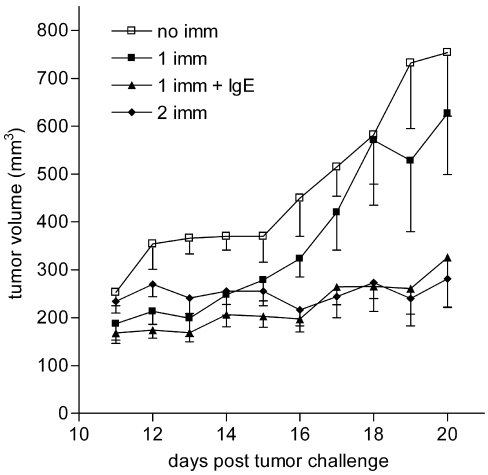

FIGURE 2. Antitumor protection induced by MVA-infected IgE-loaded RMA cells. C57BL/6 mice were s.c. vaccinated with 10^5 MVA-infected IgE-loaded (data not shown) or not loaded (2 imm) RMA cells at days −30 and −15. Other two groups of mice received only one s.c. vaccination with 10^5 MVA-infected IgE-loaded (1 imm + IgE) or IgE-free (1 imm) RMA cells at day −15. At day 0 all vaccinated mice were challenged with s.c. administration of 7×10^4 living RMA cells. Nonimmunized mice, challenged with living RMA cells have been used as controls (no imm). Results are mean ± SEM and were obtained from one of three experiments yielding similar results.

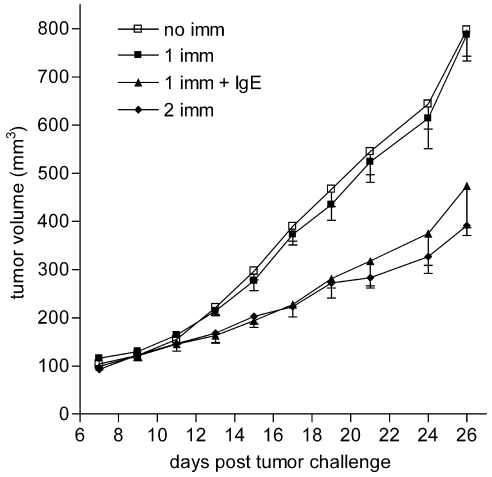

FIGURE 3. Antitumor protection induced by IgE-loaded MVA-infected TS/A-LACK cells. BALB/c mice were vaccinated s.c. with 10^5 IgE-loaded (data not shown) or IgE-free (2 imm) MVA-infected TS/A-LACK cells at day −30 and −15, or were not vaccinated (no imm). Other two groups of mice received only one s.c. vaccination with 10^5 IgE-loaded (1 imm + IgE) or IgE-free (1 imm) MVA-infected TS/A-LACK cells at day −15. At day 0 all vaccinated mice were challenged with s.c. administration of 2×10^5 living TS/A-LACK cells. Results are mean ± SEM and were obtained from one of three experiments yielding similar results.

TS/A-LACK cells ($p < 0.0001$, compared with a single immunization in the absence of IgE) (Fig. 3). However, IgE-free MVA-infected cells also induced antitumor protection after two immunizations ($p < 0.0001$, compared with a single immunization in the absence of IgE). The difference between two IgE-free immunizations and one immunization with IgE-loaded tumor cells is not statistically significant ($p = 0.4299$). Tumor growth in nonimmunized mice was similar to that in mice that received a single IgE-free immunization.

Demonstration of a key role for the IgE-FcεRI interaction in the antitumor adjuvant effect

To evaluate the relevance of IgE receptors in IgE antitumor adjuvanticity, wild-type (WT), FcεRIα$^{-/-}$, and CD23$^{-/-}$ mice were immunized with MVA-infected TS/A-LACK cells, either with or without IgE-loading. According to the results reported in Figs. 2 and 3, mice were immunized only once. Remarkably, the antitumor protection demonstrated in WT mice vaccinated by IgE-treated tumor cells was completely lost in the FcεRIα$^{-/-}$ mice. Indeed, tumor growth was not affected by the presence of IgE on the cellular vaccine ($p = 0.4470$) (Fig. 4*B*), and was comparable to that obtained in WT mice vaccinated in the absence of IgE ($p = 0.3894$) (Fig. 4, *A* and *B*). Because FcεRIα$^{-/-}$ mice still express CD23, the lack of protection indicates that CD23 alone cannot be responsible for the IgE effect. These data are the first in vivo demonstration of the crucial role played by FcεRI in the IgE-driven antitumor effect.

Conversely, CD23$^{-/-}$ mice demonstrated an unimpaired IgE effect ($p = 0.0001$, compared with a single immunization in the absence of IgE) (Fig. 4*C*). CD23$^{-/-}$ mice still express FcεRI, indicating that FcεRI is the primary (or even unique) mediator of IgE activity in antitumor vaccination.

Human IgE is an antitumor adjuvant in FcεRIα$^{-/-}$ hFcεRIα^{+} mice

Given the obvious importance of testing human IgE antitumor adjuvanticity and the relevance of the IgE-FcεRI interaction, we moved to a "humanized" system. Thus, experiments were conducted using the transgenic FcεRIα$^{-/-}$ hFcεRIα^{+} mouse (derived from the FcεRIα$^{-/-}$ mouse), in which the mouse FcεRI α-chain gene has been substituted with the human one (28).

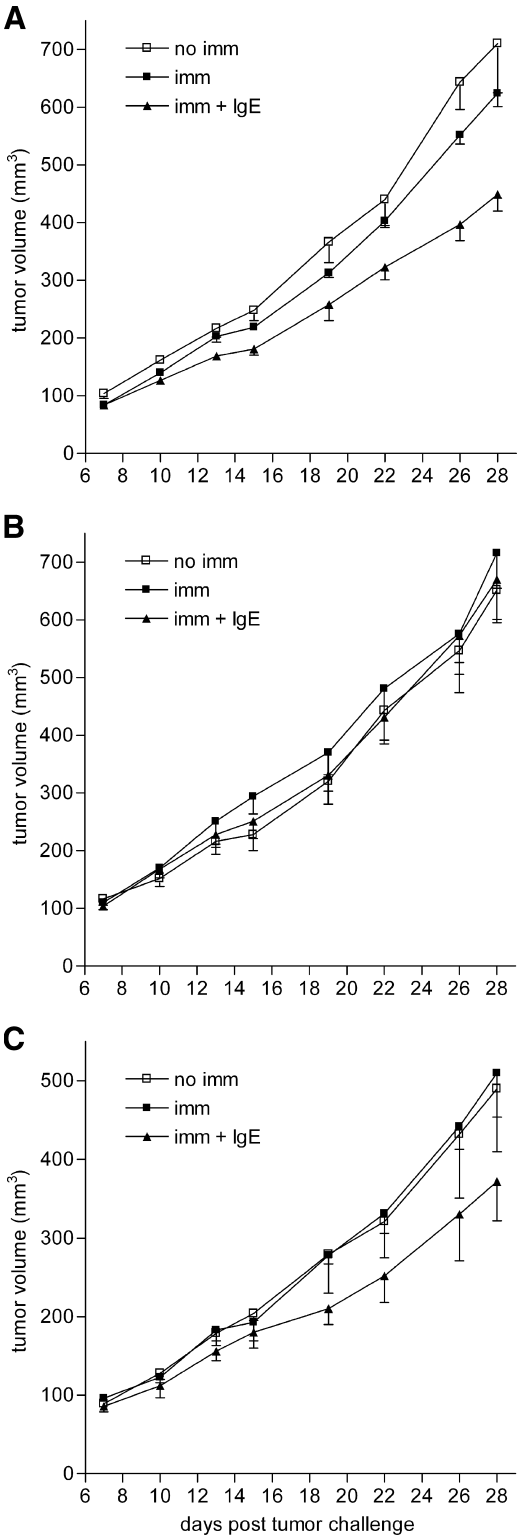

FIGURE 4. Loss of antitumor IgE adjuvant effect in FcεRIα$^{-/-}$ mice. *A*, WT mice were s.c. vaccinated with 10^5 IgE-loaded (imm + IgE) or IgE-free (imm) MVA-infected TS/A-LACK cells at day −15, or were not vaccinated (no imm). FcεRIα knockout mice (*B*) and CD23 knockout mice (*C*) were treated using the same protocol, labels are identical with previous experiments. At day 0 all vaccinated mice were challenged with s.c. administration of 2×10^5 living TS/A-LACK cells. Results are mean ± SEM and were obtained from one of three experiments yielding similar results.

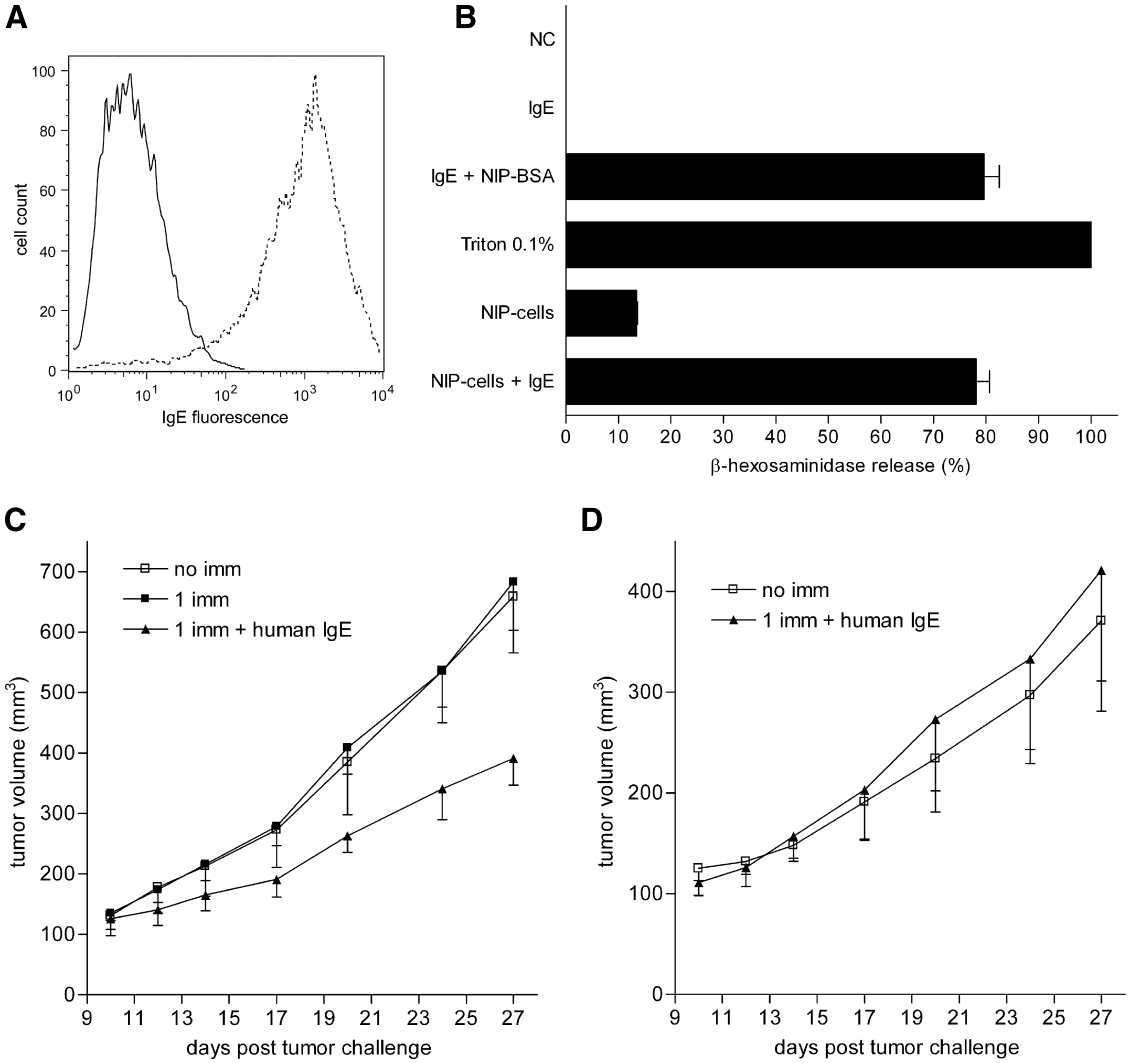

FIGURE 5. Reconstitution of antitumor IgE adjuvant effect in FcεRI$\alpha^{-/-}$ hFcεRIα^{+} mice with human IgE. *A*, Cell surface loading of human IgE. Tumor cells were haptenized using NIP-OSu and loaded with human anti-NIP IgE (dotted histogram) or not loaded (thin line histogram). IgE loading was verified by cytofluorimetric analysis using biotinylated anti-human IgE followed by streptavidin-PE. *B*, IgE-mediated β-hexosaminidase release assay. RBL-SX38 cells were put in contact with human IgE-loaded (NIP-cells + IgE) or not loaded (NIP-cells) haptenized tumor cells. A 100% release corresponds to the release obtained by lysing cells with 0.1% Triton X-100. Positive control consists of anti-NIP soluble IgE-sensitized RBL-SX38 cells cross-linked with NIP-BSA (IgE + NIP-BSA). Negative controls (NC): nonstimulated RBL-SX38 cells; anti-NIP soluble human IgE-loaded RBL-SX38 cells in the absence of NIP-BSA (IgE). Results are mean ± SD of three determinations. *C*, FcεRI$\alpha^{-/-}$ hFcεRIα^{+} mice were s.c. vaccinated with 10^5 human IgE-loaded or human IgE-free MVA-infected TS/A-LACK cells at day −30 and −15 (data not shown). Other two groups of mice received only one s.c. vaccination with 10^5 human IgE-loaded (1 imm + human IgE) or human IgE-free (1 imm) MVA-infected TS/A-LACK cells at day −15. At day 0, all vaccinated mice were challenged with s.c. administration of 2×10^5 living TS/A-LACK cells. Nonimmunized mice, challenged with living TS/A-LACK cells have been used as controls (no imm). *D*, As a control of the experiment in *C*, BALB/c WT mice were s.c. vaccinated with 10^5 human IgE-free MVA-infected TS/A-LACK cells at day −30 and −15 (data not shown) or with 10^5 human IgE-loaded (1 imm + human IgE) MVA-infected TS/A-LACK cells at day −15. At day 0 all vaccinated mice were challenged with s.c. administration of 2×10^5 living TS/A-LACK cells. Nonimmunized mice, challenged with living TS/A-LACK cells have been used as controls (no imm). Results are mean ± SEM and were obtained from one of three experiments yielding similar results.

MVA-infected TS/A-LACK cells were haptenized with NIP, loaded with an anti-NIP chimeric human IgE mAb and loading was verified by FACS analysis (Fig. 5*A*). Contact between human IgE-loaded tumor cells and the basophilic cell line RBL-SX38, expressing human FcεRI (29), led to cell degranulation and massive release of β-hexosaminidase (Fig. 5*B*). As with the mouse IgE setup (Fig. 1), this mediator release was clearly dependent on the presence of cell surface IgE. Mice were vaccinated either once or twice (15 days apart) with MVA-infected TS/A-LACK cells (with or without human IgE-loading) and challenged 15 days after the last immunization. Mice immunized once with IgE-free MVA-infected cells were not protected, showing a tumor development comparable to that of nonimmunized control mice ($p = 0.5777$). Most importantly, as in the WT model with mouse IgE, a single immunization in the presence of human IgE provided the expected adjuvant effect ($p < 0.0001$ compared with a single immunization in the absence of IgE) (Fig. 5*C*). The degree of antitumor protection was comparable to that obtained with two immunizations in the absence of IgE ($p = 0.1396$; data not shown). As controls for the experiment, WT mice (i.e., expressing mouse FcεRI) were immunized twice with MVA-infected TS/A-LACK cells, immunized once with human IgE-loaded MVA-infected TS/A-LACK cells or not immunized. As expected, two immunizations protected mice from tumor development ($p = 0.0001$ compared with no immunization; data not shown). The

single immunization in the presence of human IgE did not affect tumor growth, which was comparable to that observed in non-immunized mice ($p = 0.2398$) (Fig. 5*D*). These results provide further evidence that the antitumor effect is IgE-mediated via the interaction with FcεRI because human IgE does not bind murine FcεRI (42). The reconstitution of the IgE effect in the FcεRIα$^{-/-}$ hFcεRIα^{+} mouse represents the first demonstration of human IgE antitumor adjuvanticity.

Discussion

In the last 20 years, many studies have described IgE-related antitumor effects (43). However, an endogenous IgE with TAA specificity derived from cancer patients or healthy individuals is still awaiting to be discovered, although initial efforts in this direction are being made (44).

The ability of mouse IgE to affect tumor growth and establish an antitumor immunological memory in C57BL/6 mice has been reported (25). In that study, IgE tumor cell loading was obtained by a three-step strategy, based on a biotin-avidin bridge. The vaccination protocol consisted of two 15 day-spaced immunizations with IgE- or IgG-loaded irradiated tumor cells, followed by a challenge with living tumor cells. IgG loading did not show any statistically significant difference in tumor protection as compared with the effect obtained by immunizing mice with Ig-free tumor cells (our unpublished observation). Conversely, mice vaccinated with IgE-loaded cells showed a powerful tumor protection, indicating that IgE can exert an adjuvant effect. The involvement of eosinophils, CD4^{+} T cells and CD8^{+} T cells appeared crucial because the depletion of any of these cell types abrogated tumor protection.

In the present work, we further investigated IgE antitumor adjuvanticity by probing the involvement of FcεRI. The vaccination protocol was modified to eliminate the dependence on specific TAAs and, according to the above-mentioned evidence, an IgG-driven antitumor effect was not taken into consideration. IgE loading was attained by cell surface haptenization and hapten-specific IgE targeting, making the strategy suitable for any tumor, including those less characterized for antigenicity, as well as the less immunogenic ones. Along these lines, the BALB/c mammary adenocarcinoma cell line TS/A, whose LACK-transfected version was successfully used in this work, falls in the category of the poorly immunogenic tumors (45). Furthermore, mice vaccination was established with MVA-infected tumor cells, heading toward a viral vector-based vaccine development (see below). MVA is an attenuated VV derivative (39), unable to replicate in mammalian cells, currently used for the clinical development of therapeutic cancer vaccines (40). Interestingly, the protective IgE effect was established after a single vaccination with IgE-loaded MVA-infected tumor cells. Most likely, the immunogenicity of MVA potentiates IgE adjuvanticity because in the protocol using irradiated IgE-loaded tumor cells, two immunizations were necessary to attain the IgE antitumor effect (25).

It is conceivable that the IgE adjuvanticity observed in antitumor vaccination could result from an inflammatory reaction, similar to those induced by IgE in allergic manifestations. Because such reactions are mediated by FcεRI activation, investigating the dependence of the IgE antitumor effect upon FcεRI activation was of utmost importance. To this aim, the availability of BALB/c FcεRIα$^{-/-}$ and CD23$^{-/-}$ mice was exploited. First, the IgE protective effect was confirmed in a BALB/c tumor model, where IgE-loaded MVA-infected tumor cells proved to be an effective cellular vaccine. Second, FcεRIα$^{-/-}$ mice vaccinated with IgE-loaded MVA-infected tumor cells did not show any antitumor protection, demonstrating that FcεRI plays a crucial role in vivo in IgE adjuvanticity. Third, CD23$^{-/-}$ mice vaccinated with IgE-loaded MVA-infected tumor cells showed antitumor protection (27), indicating that CD23 is not involved in the IgE adjuvant effect and reinforcing the unique importance of FcεRI. Yet, in an in vitro cellular assay, CD23 was found to contribute to a direct IgE-mediated tumor cell killing via Ab-dependent cellular phagocytosis (20, 21). Ab-dependent cellular phagocytosis mediated by the IgE-CD23 interaction could be significant at the tumor site but may not contribute in the development of an antitumor immune response, thus justifying the differences of CD23 relevance in the two protocols. The identification of the essential role played by the IgE-FcεRI interaction constitutes an important insight, allowing further investigations on the molecular and cellular mechanisms that build the observed antitumor effect.

Next, the need for the implementation of a human IgE system paralleling the mouse IgE system becomes of primary importance. Therefore, the effect of a human IgE-loaded MVA-infected tumor cell vaccine was studied in WT and FcεRIα$^{-/-}$ hFcεRIα^{+} (28) BALB/c mice. Human IgE had no effect in WT BALB/c mice, but induced a significant protection in the humanized knock-in mouse. This result is the first demonstration of an adjuvant effect exerted by human IgE in antitumor vaccination. FcεRIα$^{-/-}$ hFcεRIα^{+} mice present a cellular distribution of the receptor similar to that observed in humans (28, 46, 47). Human FcεRI expression is not confined to mast cells and basophils, as in mice, but it has been shown also in dendritic cells, eosinophils, monocytes, and platelets (1, 42). Therefore, the FcεRIα$^{-/-}$ hFcεRIα^{+} mouse represents an ideal experimental system to study the human IgE effect.

The results reported in this study strongly suggest that the IgE adjuvant effect in antitumor vaccination is mediated by the IgE-FcεRI interaction in a scenario characterized by the contact between IgE-loaded tumor cells and FcεRI^{+} cells. Subsequently, tumor cells could be killed directly by FcεRI^{+} cells or indirectly through effector cells recruited by inflammatory signals released from FcεRI^{+} cells. Cellular killing could lead to tumor Ag presentation by dendritic cells to peripheral immune districts with the consequent instauration of a tumor-specific T cell immunological memory. Overall, this work embodies a major step forward in the development of an IgE-based cellular vaccine, together with a better understanding of the in vivo importance of FcεRI involvement. The exploitation of the knowledge on the IgE-FcεRI interaction (1, 30, 42, 48), together with the experimental systems established in this study, should allow further progress in the application of IgE adjuvanticity. The proof of principle that surface IgE is a powerful adjuvant in antitumor immunity has been established. Therefore, we are now aiming at producing recombinant MVA that could induce the expression of membrane IgE on the surface of infected cells as well as recombinant MVA expressing both IgE and TAA to be tested as antitumor viral vaccines. Finally, the establishment of a human IgE model system in mouse represents a fundamental resource for future developments in IgE antitumor vaccination, moving the field closer to the clinics.

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Disclosures

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