

Comparison of IgE and IgG antibody-dependent cytotoxicity *in vitro* and in a SCID mouse xenograft model of ovarian carcinoma

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Allergic reactions are mediated by IgE antibodies bound to high-affinity receptors on mast cells in peripheral tissues and are characterized by their immediacy and hypersensitivity. These properties could also be advantageous in immunotherapy against cancer growth in peripheral tissues. We have constructed chimeric IgE and IgG1 antibodies with murine V regions and human C regions corresponding to the MOv18 monoclonal antibody against the human ovarian tumor-associated antigen, folate binding protein. The antibodies exhibited the expected binding affinities for antigen and Fc receptors, and effector activities with human basophils and platelets *in vitro*. The protective activities of MOv18-IgE and MOv18-IgG1 were compared in a SCID mouse xenograft model of ovarian carcinoma. The beneficial effects of MOv18-IgE were greater and of longer duration than those of MOv18-IgG1. Our results suggest that the allergic reaction could be harnessed for the suppression of ovarian tumors.

Key words: Allergy / Folate binding protein / IgE / Immunotherapy / Ovarian cancer

Received	19/5/99
Revised	22/7/99
Accepted	22/7/99

1 Introduction

Adoptive therapy with mAb of the IgG1 class is successful against lymphomas [1–3], but of limited efficacy in the treatment of tumors of non-hematopoietic origin [4]. The basis for the choice of Ab class in lymphoma therapy was the observation that IgG1 is more effective than other Ab classes in antibody-dependent cell-mediated cytotoxicity (ADCC) against tumors *in vitro* [5–7]. *In vivo* comparisons of anti-tumor effects by different Ab classes have been confined to only IgG and IgM in both murine models [8] and lymphoma patients [2]. IgE and IgG Ab have not been compared for immunotherapeutic efficacy in either human patients or murine models.

IgE Ab are known primarily as the agents of allergy [9]. Atopy has been linked to increased resistance to infection by certain types of parasites, but no other beneficial function is known [9]. Allergic rhinitis and asthma are characterized by immediate hypersensitivity, followed by an inflammatory reaction requiring the recruitment of effector cells from the circulation into the tissue. The uniqueness of the allergic reaction is due to the presence in the tissue of mast cells and Langerhans cells that are essentially permanently sensitized by IgE Ab bound to the high-affinity IgER, FcεRI. The cross-linking of FcεRI on mast cells by allergen leads to the release of histamine and cytokines, which increase circulation to the tissue and lead to the influx and activation of inflammatory and potentially cytotoxic cells at the site of allergen provocation. Allergen-activated Langerhans/dendritic cells migrate to local lymph nodes and stimulate cognate T cells, which in turn migrate to the tissue and participate in the inflammatory response and the stimulation of Ab synthesis. We hypothesize that IgE-targeted immediate

[1 19675]

Abbreviations: ADCC: Antibody-dependent cell-mediated cytotoxicity FBP: Folate binding protein

hypersensitivity reactions and allergic inflammation might be harnessed for the killing of malignant cells in tissues. Given that allergic reactions are largely dependent on sensitized mast cells and Langerhans cells, which are confined to tissues, the previously reported inactivity of IgE relative to IgG1 in ADCC assays *in vitro* [6] may underestimate the potency of IgE.

If protection against cancer were a natural function of IgE, one might predict that individuals with allergies would have a lower risk of cancer. A relation between allergy and cancer has been sought in a number of epidemiological studies. Many of the early studies indeed appeared to demonstrate an inverse correlation between allergy and the risk from cancer [10–15]. However, this generality was not borne out in later studies based on larger groups of patients [16, 17]. In a retrospective study, Mills et al. [16] concluded that the association between allergy and cancer is complex and depends on the specific allergy and specific organ site. Allergies appeared to increase the risk of developing some forms of cancer, notably, myelomas and sarcomas. The only statistically significant result in this study, however, was the inverse correlation between the incidence of allergies and ovarian cancer [16]. To investigate this association further, we first sought a suitable ovarian tumor-associated antigen as a potential target for an IgE Ab-mediated effect.

The folic acid receptor, also known as folate binding protein (FBP), is expressed on the cell surface of the majority of malignant as well as benign non-mucinous epithelial ovarian tumor cells, and the levels of FBP are related to clinical severity [18, 19]. FBP occurs at high densities (10^6 molecules/cell) on ovarian tumor cells, but not on normal cells, and is thus an attractive target for therapy [20]. An mAb named MOv18, which recognizes an epitope on FBP, has been generated and extensively characterized [21, 22]. A chimeric MOv18-IgG1, with mouse variable and human constant regions, directs ADCC against the human ovarian cell line IGROV1 [23] by human PBMC *in vitro* [20]. A bispecific Ab containing MOv18 Fab and anti-CD3 Fab units has also been used to target cytotoxic T cells to ovarian tumor cells *in vitro* [24], as well as *in vivo* in a mouse xenograft model [25] and in human patients [26]. I.p. injection of *in vitro* activated T cells, bearing the bispecific Ab, into patients destroyed peritoneal cancer cells, but tumors later developed in the retroperitoneal lymph nodes [26]. Expression of a chimeric Ab with tandem MOv18 light- and heavy chain V-regions attached to a membrane anchor in tumor-infiltrating lymphocytes conferred cytotoxicity against IGROV1 cells *in vitro* [27]. MOv18 permitted localization and ^{131}I targeting to human ovarian tumor xenografts in nude mice [28], pointing to its applications in radioimaging and radioimmunotherapy;

so far, however, attempts at radioimmunotherapy in the treatment of ovarian cancer patients have met with only modest success [29, 30].

Our aim here was to compare the efficacies of chimeric IgE and IgG1 Ab of the same specificity in a severe combined immunodeficiency (SCID) mouse model of an appropriate tumor target. The IGROV1 cell line was the target of choice for the following reasons: (1) epidemiological data specifically suggested a natural function for IgE in immune defense against ovarian tumors; (2) ovarian carcinomas are relatively common and usually fatal, even with optimal existing therapies [31]; (3) a specific antigen (FBP) has been identified and the genes for a chimeric Ab (MOv18-IgG1) [20] and for the ϵ chain of IgE [32] have been cloned; (4) the efficacy of MOv18 in targeting to ovarian tumor cells *in vitro* and *in vivo* was well established; and (5) IGROV1 cells provided a suitable target for studies *in vitro* and *in vivo* in SCID mice.

2 Results

2.1 Preparation and characterization of the Ab

To express the chimeric MOv18-IgE, we first constructed a chimeric MOv18 ϵ chain expression vector, encoding the murine MOv18 heavy chain variable region (V_H) and the human ϵ chain constant region ($C\epsilon$). The chimeric MOv18 ϵ chain vector and the chimeric MOv18 κ chain vector, previously used to express the chimeric MOv18-IgG1 [20], were co-transfected into a Chinese hamster ovary (CHO) cell line, and stable lines secreting the IgE Ab were selected. We also expressed a chimeric IgE corresponding to the hybridoma Ab, SF25, which recognizes an antigen unrelated to FBP on colon carcinoma cells [33], to use as a control for antigen specificity. The method of construction of the chimeric κ chain expression vectors has been described in previous work [20].

MOv18-IgE and SF25-IgE were purified from the culture supernatants by affinity chromatography, using an anti-IgE-Fc mAb. Electrophoresis of the affinity-purified MOv18-IgE (Fig. 1A, B) and SF25-IgE (results not shown) on SDS-polyacrylamide gels under nonreducing and reducing conditions (Fig. 1A) and HPLC analysis (Fig. 1B) revealed that the products are monodisperse, with the expected subunit composition and molecular mass. Western blotting confirmed the identities of the isotypes of the heavy and light chains (results not shown).

To target cytotoxic cells to tumors, the chimeric IgE must have high affinity for IgER on the former, and for antigen on the latter. The affinities of the MOv18-IgE for

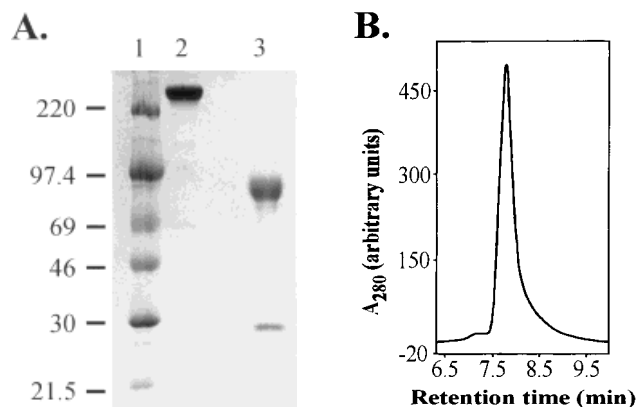


Figure 1. Purity of MOv18-IgE. (A) SDS-polyacrylamide gradient gel electrophoresis profile of purified MOv18-IgE. Lane 1, molecular mass markers (kDa); lane 2, Ab under nonreducing conditions; lane 3, Ab under reducing conditions. (B) HPLC analysis of purified MOv18-IgE.

FBP on IGROV1 cells and for IgER were accordingly measured. In earlier work using the chimeric MOv18-IgG1, we found that the level of expression of FBP on ovarian tumor cells was an important factor in determining their susceptibility to ADCC *in vitro* and that cells of the IGROV1 human ovarian tumor cell line provided a suitable target cell [20]. In the present work with MOv18-IgE, we obtained an estimate of 8×10^5 molecules of FBP per IGROV1 cell and a dissociation constant (K_D) of 4×10^{-9} M, in good agreement with the values previously found for MOv18-IgG1 [20]. The association and dissociation rate constants for MOv18-IgE binding to Fc ϵ R1 on a transfected CHO cell line were $k_{+1} = 2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{-1} = 2 \times 10^{-5} \text{ s}^{-1}$, respectively, leading to a dissociation constant of the expected magnitude ($K_D = 10^{-10}$ M) [34]. SF25-IgE was found to have similar affinity for Fc ϵ R1.

2.2 *In vitro* activities of the Ab

The capacity of MOv18-IgE to sensitize effector cells for activation by IGROV1 cells was tested with isolated human basophils and platelets, known to express Fc ϵ R1. Basophils, sensitized with either MOv18-IgE or anti-NP IgE, released histamine on anti-IgE activation, with a higher response approximately 60 % of total histamine released) and greater sensitivity to the stimulus than control cells (Fig. 2 A). Moreover, IGROV1 cells were clearly able to induce significant histamine release (over that occurring spontaneously) from basophils sensitized with MOv18-IgE (Fig. 2 B), but not when sensitized with either anti-NP-IgE or MOv18-IgG1. It follows that the MOv18-IgE-IGROV1 interaction, which leads to basophil degranulation, is a specific response, not engendered by

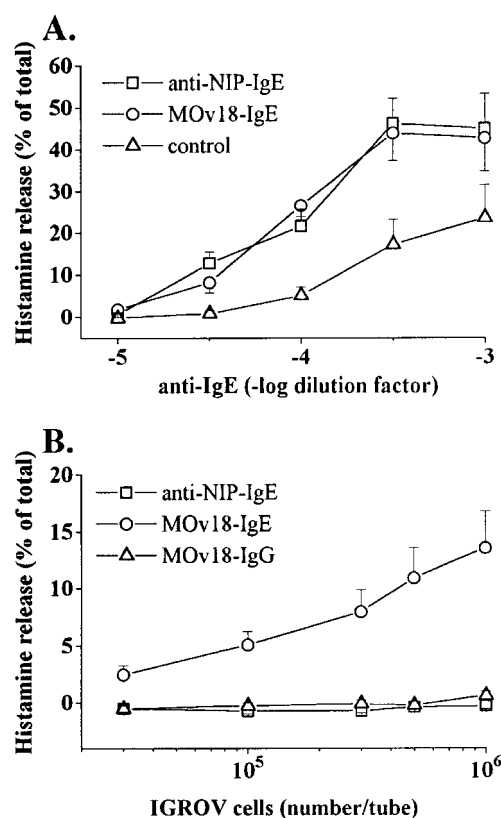


Figure 2. Histamine release from basophil leukocytes induced by (A) anti-IgE Ab or (B) IGROV1 cells. Before activation basophils were incubated with the Ig specified for 1 h, whilst control cells, to assess the effectiveness of the acid stripping procedure, were incubated in buffer alone. Histamine release is expressed as a percentage of total cellular histamine. Results are means $\pm$ SEM of four or five experiments carried out using cells from four blood donors.

the action of another chimeric IgE (anti-NP) or the IgG1 subclass of the specific Ab.

MOv18-IgE-dependent stimulation of normal human platelets was assessed against bystander schistosomes, serving as targets of cytotoxic mediators [35]. Specific ligands of surface MOv18-IgE, whether anti-IgE or IGROV1 cells, induced killing of parasite larvae (Fig. 3 A) at levels comparable to those found with parasite-specific IgE or with atopic IgE and specific allergen. SF25-IgE and MOv18-IgE induced significantly greater killing of schistosome larvae when combined with cells bearing the cognate antigen, LS-180 cells and IGROV1 cells, respectively, demonstrating the importance of antigen recognition. Preincubation of platelets with increasing concentrations of Fab fragments from anti-human Fc ϵ R1 mAb 15-1 [36] significantly inhibited the MOv18-IgE-induced cytotoxic functions of platelets, implicating the high-affinity IgER and excluding a role for the low-

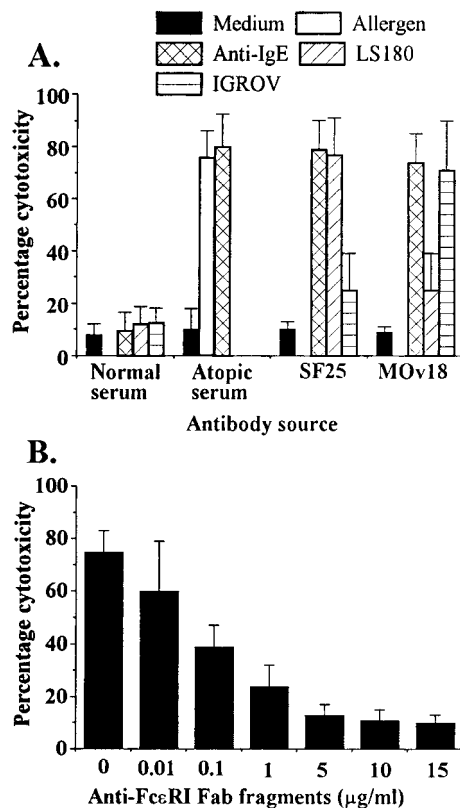


Figure 3. The IgE- and FcεRI-dependent cytotoxicity mediated by human platelets. (A) IgE-dependent killing of bystander schistosomules by human platelets. IgE was provided by sera from patients with allergy (atopic) by MOv18-IgE or SF25-IgE. Normal human serum was included as a negative control. IgE cross-linking was achieved by cells bearing antigen (IGROV1, LS-180), specific allergen (*Der p 1*) or anti-IgE Ab as indicated. (B) MOv18 and IGROV1 induced bystander schistosomule cytotoxicity is FcεRI dependent. Experiments were carried out in a similar way as those described above. However, prior to the addition of the MOv18-IgE and IGROV1 cells, platelets were incubated for 30 min with increasing concentrations of 15–1 Fab fragments.

affinity IgER (FcεRII/CD23) in mediating the effector function of MOv18-IgE (Fig. 3B).

When MOv18-IgE, SF25-IgE or MOv18-IgG1 were incubated with ^{51}Cr -labeled IGROV1 cells and human lymphocytes at an E/T ratio of 50:1 for 4–16 h, between 40 and 80 % lysis occurred with the IgG1 and none with the IgE Ab. LS-180 cells, which do not express FBP, exhibited no Ab-dependent cell lysis with either MOv18-IgE or MOv18-IgG1 (Table 1). Similar results were obtained with PBMC (results not shown). Thus, only IgG1 in combination with the appropriate target cell induced ADCC *in vitro* under the conditions of these experiments.

Table 1. IgG1-dependent killing of IGROV1 cells by human PBL *in vitro*^{a)}

	% ^{51}Cr release	
	IGROV1 cells	LS180 cells
Spontaneous release	8	5
PBL alone	9	23
Mov18-IgG1	7	6
MOv18-IgE	11	4
Mov18-IgG1 + PBL	50	25
MOv18-IgE + PBL	11	18

a) A typical experiment in which the E/T ratio was 50:1 and ^{51}Cr release was measured at 5 h is shown. The LS-180 cells were susceptible to NK cell killing (PBL alone values), but not antibody-directed killing. Duplicate samples had an error of < 3 %.

2.3 *In vivo* activities of the Ab

The capacity of MOv18-IgE to recruit effector cells against an IGROV1 tumor challenge *in vivo* was tested in a SCID xenograft mouse model of ovarian cancer. SCID mice were pretreated with rabbit anti-asialo G_{M1} Ab i.p. to eliminate host NK cell resistance to the human donor cells. One day later (day 1), all mice received an s.c. challenge in the flank with 2.5×10^6 IGROV1 tumor cells, followed by i.v. administration of 3×10^6 human PBMC into three of the four experimental groups. Either MOv18-IgE (50 and 100 μg) or MOv18-IgG1 (100 μg) was then injected i.v. into the animals in the PBMC groups (Fig. 4A). The average tumor size in the control group, given only IGROV1 cells, exceeded 200 mm² by day 35 after challenge. Both MOv18 IgE and IgG1 exhibited a dramatic inhibition of tumor growth up to day 19, but IgG1 had no effect thereafter. Tumor growth was similar in untreated mice and those injected with PBMC and MOv18-IgG1 ($p > 0.96$) at day 35. In contrast, mice given PBMC and 50 μg MOv18-IgE exhibited reduced growth by both day 29 (51 % inhibition; $p < 0.03$) and day 35 (40 % inhibition; $p < 0.04$). Mice injected with 100 μg MOv18-IgE exhibited an even greater degree of inhibition, relative to the positive tumor control, on day 29 (72 % inhibition; $p < 0.001$) and day 35 (62 % inhibition; $p < 0.005$). In the absence of human PBMC MOv18-IgE did not significantly inhibit tumor growth (results not shown), demonstrating the requirement for human cells to mediate the antigen-specific IgE effector function. These results imply that only the IgE-based chimeric Ab, allied with human cells, was effective in mediating an anti-tumor response lasting at least 35 days.

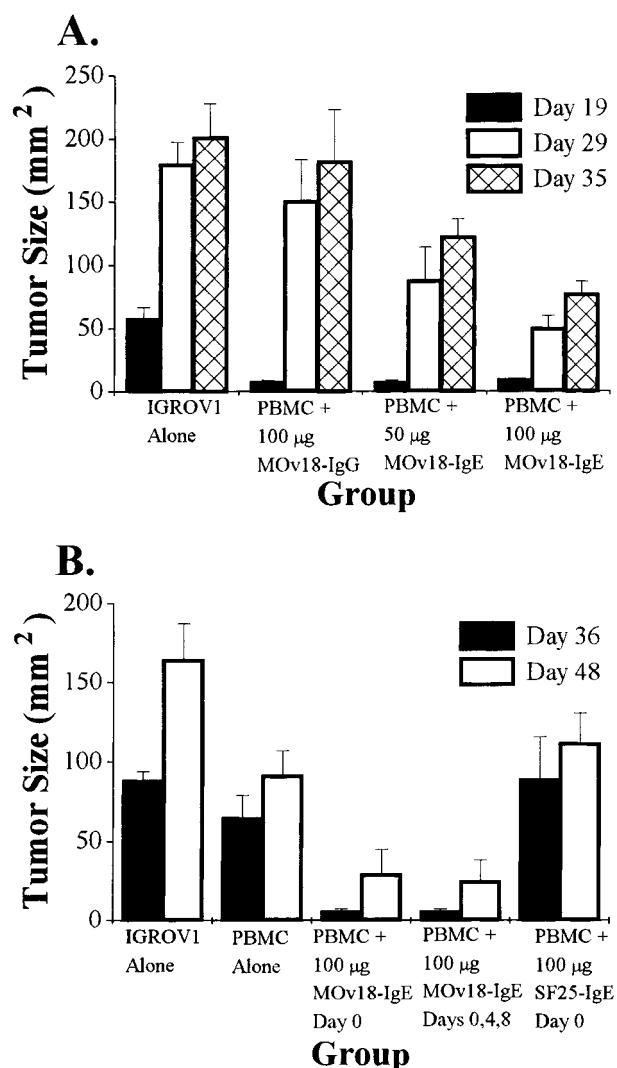


Figure 4. Inhibition of IGROV1 cell growth in SCID mice. (A) Comparison of the effects of MOv18-IgE and MOv18-IgG1. Either MOv18-IgE (50 and 100 µg) or MOv18-IgG1 (100 µg) was injected i.v. into the PMBC groups. The mean tumor size $\pm$ SEM was measured on days 19, 29 and 35 after challenge ($n = 4$ for all groups). (B) Comparison of the effects of specific and nonspecific isotype-matched Ab. Mice were prepared and analyzed as above, but PBMC were administered either alone ($n = 5$), with 100 µg MOv18-IgE ($n = 5$), with multiple injections of 100 µg MOv18-IgE on days 0, 4 and 8 ($n = 5$), or with 100 µg SF25-IgE ($n = 4$); $n = 4$ for the positive control group given tumor alone. The mean tumor size $\pm$ SEM was measured on days 36 and 48 after challenge.

The specificity of this response was shown by the lack of tumor growth inhibition, beyond that caused by PBMC alone, following injection of PBMC with 100 µg SF25-IgE (Fig. 4 B). In this experiment a single injection of MOv18-IgE with PBMC on day 1 reduced tumor size on day 48

by 83 % ($p < 0.001$), compared to 45 % after injection of PBMC alone ($p < 0.06$).

Tumors sampled on day 35 from the experiment described in Fig. 4 B were stained with hematoxylin and eosin. Tumors revealed moderately to poorly differentiated epithelial malignancies growing as nodules with subcutaneous murine tissue. Viable tumor cells revealed the presence of intact, anaplastic nuclei and the formation of sheets and abortive glands; necrotic cells contained either minute nuclear fragments or undetected nuclei and were localized to sharply demarcated regions without discernible cytoarchitecture. Image analysis revealed that tumors from mice that received only PBMC or PBMC + SF25-IgE showed significantly less necrosis in randomly selected fields (17.4 % and 15 % of the tumor area, respectively), compared with those from mice that received PBMC + MOv18-IgE (41.6 %; $p < 0.001$; Fig. 5 A). Direct visualization of representative sections from the tumors of mice receiving PBMC alone (Fig. 5 B) or PBMC + MOv18-IgE (Fig. 5 C) clearly confirmed this result, demonstrating the beneficial effect of treatment with MOv18-IgE.

3 Discussion

We postulated that IgE may have advantages over IgG antibodies in the rejection of ovarian tumor cells *in vivo*. To test this, we have expressed and purified the chimeric IgE Ab, MOv18-IgE, containing murine V regions directed against FBP and human C κ and C ϵ regions. We have compared the activities of this antibody with those of the corresponding MOv18-IgG1, generated in a previous study [20]. MOv18-IgE binds FBP on IGROV1 cells and IgER (Fc ϵ RI and Fc ϵ RII/CD23) with native affinity. In the presence of IGROV1 cells, MOv18-IgE mediated the activation of basophils to release histamine and platelets to release substances cytotoxic for bystander schistosome larvae. MOv18-IgG1 lacked these particular activities, but did mediate IGROV1 cytolysis by PBMC, in contrast to MOv18-IgE under the same conditions. These results confirmed earlier observations with a set of eight murine Ab, differing only in the Ab class, which demonstrated IgG, but not IgE ADCC activity *in vitro* [6].

In support of our hypothesis, the results of functional assays *in vivo* exhibited the inverse relationship between IgE and IgG1 forms of MOv18. Although both MOv18-IgE and MOv18-IgG1 (50–100 µg i.v. with 2.5×10^6 human PBMC on day 0) inhibited the growth of IGROV1 (2×10^6 cells s.c. on day 0) in SCID mice for a significant length of time (up to 19 days), only MOv18-IgE sustained inhibition of tumor growth until the end of the experiments (35 or 48 days). The inhibitory effect of

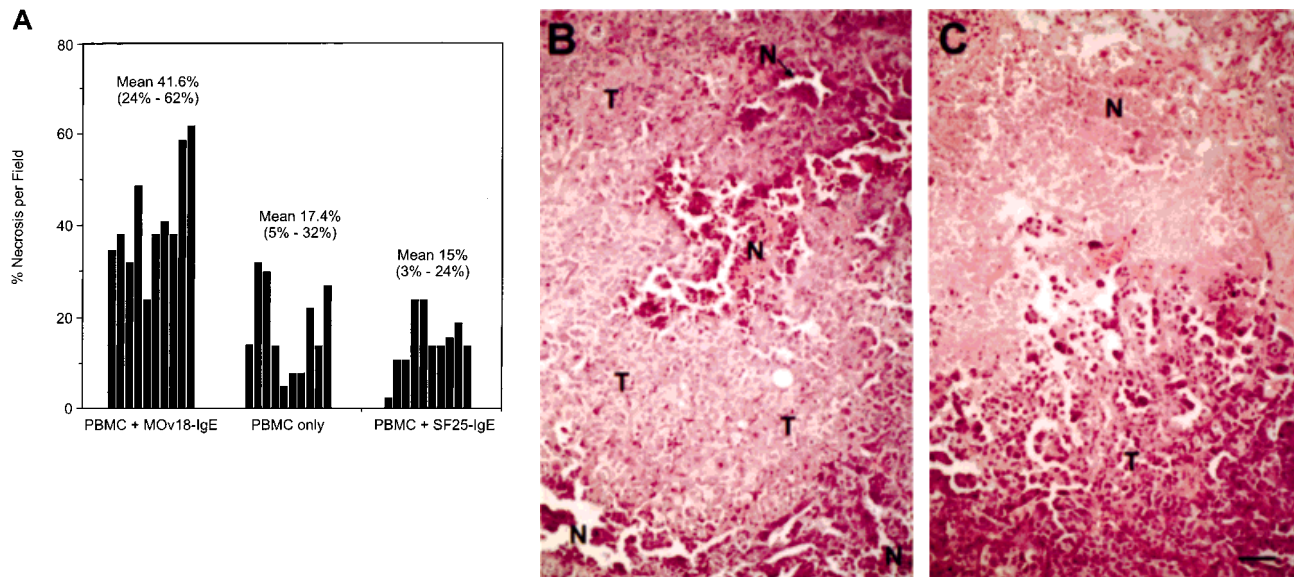


Figure 5. Histologic examination of IGROV1 tumors. (A) Image analysis of hematoxylin and eosin-stained tumor sections from mice treated with MOv18-IgE + PBMC, SF25-IgE + PBMC or PBMC alone. This demonstrates the antigen specificity of ovarian tumor necrosis induced by treatment with MOv18-IgE. The mean % necrosis for the PBMC + MOv18-IgE group was 41.6 ± 3.8 % SEM, as compared to $17.4 \pm$ SEM for the PBMC alone ($p < 0.001$) and 15.0 ± 2.0 % for PBMC + SF25-IgE ($p < 0.001$). Direct visual examination of representative tumor sections from mice treated with PBMC alone (B) and from mice treated with PBMC + MOv18-IgE (C) reveals the significantly greater area of tumor necrosis resulting from MOv18-IgE treatment. Scale bar 72 μ m. T: tumor areas; N: necrotic areas.

MOv18-IgE was substantial and highly significant, *i.e.* 72 % ($p < 0.001$) and 83 % ($p < 0.001$) at these times. While the tumors grew to some extent in all the mice in these experiments, the optimal protocols for treatment of the mice (*e.g.* dose, frequency and timing of administration of Ab and PBMC) have not yet been determined, and may well further diminish tumor growth.

Since tumor growth inhibition required co-injection of human PBMC with the MOv18-IgE, and since human IgE does not bind to rodent IgER [37], our results imply that human cells mediated IgE Ab-dependent IGROV1 cell killing in the SCID mice. This could occur by direct IgE-ADCC to IGROV1 cells, perhaps mediated by macrophages or other Fc ϵ RI-expressing cells. Alternatively, human effector cells, stimulated by IGROV1 cells, may release chemoattractants and/or cytokines to recruit and activate SCID mouse cytolytic effector cells in a bystander fashion. Further work is in progress to determine the mechanism of tumor killing.

We observed that the activity of MOv18-IgE and IgG1 differs in the duration, as well as the final extent of tumor growth inhibition. These results suggest that retention of the Ab at the tumor site may be an important factor in determining its efficacy and reflect the affinities of IgE

and IgG for their FcR. The intrinsic affinity of IgE for Fc ϵ RI (10^{10} M $^{-1}$) is at least two orders of magnitude higher than that of IgG Ab for IgGR (10^6 – 10^8 M $^{-1}$) [37]. The association constants mainly reflect the off-rates, which in the case of the IgE-Fc ϵ RI interaction is exceptionally slow ($t_{1/2} \sim 16$ h, measured in binding assays with cells in suspension) [34]. In tissues, the release of IgE from cells is considerably slower, owing to restricted diffusion and re-binding of IgE to FcR. We have measured the half-life of IgE in skin to be of the order of 2 weeks [38]. Thus MOv18-IgE Ab-dependent effector functions at the s.c. sites of IGROV1 cells in our experiments would be expected to exert anti-tumor effects for several weeks, while the IgG1 activity would decay much faster, as in fact observed.

One of the potential problems in immunotherapy with IgE might be systemic anaphylaxis if, for example, the tumor shed antigen into the circulation. We observed no signs of anaphylaxis in the SCID mice, despite the fact that FBP is indeed shed from cell membranes [39]. This was not entirely unexpected, since any released FBP would be monovalent and hence unable to cross-link the IgER complexes on cells required for activation. In addition, the humanized MOv18-IgE is incapable of binding to murine IgER on potential reactive cells [37]. Neverthe-

less, the lack of systemic anaphylaxis was also conspicuous in another study with a murine IgE antibody against a viral tumor antigen [40]. Recent findings may explain why tumor regression, but not systemic anaphylaxis, would likely occur. IgE concentrations in serum ($\sim 10^{-11}$) are well below the threshold ($\sim 10^{-7}$ M) required to induce Fc ϵ R1 expression on circulating IgE effector cells [41]. These considerations suggest that IgE antibodies may be safe for use in humans if serum concentrations of IgE are kept well below 10^{-7} M.

The application of IgE to cancer therapy was previously advanced by Sehon and co-workers [40], who reported that a murine IgE Ab against mammary tumor virus protected mice against tumor growth. However, IgE was not compared with any other Ab class in this work. We would expect IgE to be much more efficient in human patients than in the SCID mouse model. Human PBMC disappear very rapidly from the circulation of SCID mice and are widely distributed amongst various tissues [42], while in humans the supply of effector cells would be constant.

The epidemiological results of Mills et al. [16] suggested that IgE could have a natural function in the early recognition and killing of ovarian tumor cells. The corollary, that IgE may be useful in immunotherapy of ovarian carcinoma, is supported by our results. The implication, which may now be tested, is that similar mechanisms operate in the allergic response and in IgE-mediated rejection of tumor cells in peripheral tissues. The xenograft model of human ovarian carcinoma may underestimate expectations from the clinical application of IgE in patients with minimum residual disease following surgery.

4 Materials and methods

4.1 Cloning the MOv18 and SF-25 chimeric ϵ heavy chain expression vectors

The expression vector for chimeric MOv18-IgG1, pSV2 Δ gptMOv18V_H-hC γ 1 [20], was digested with Sal I, end-trimmed with mung bean nuclease, recut with BamH I, and end-filled with Klenow DNA polymerase (Step 1 in Fig. 6). These procedures yielded a blunt-ended 9.75-kb fragment, which contained pBR322, a gpt marker and the MOv18 V_H gene. The fragment was circularized to regenerate the BamH I site downstream of the V_H gene in pSV2 Δ gptMOv18V_H. To insert the human C ϵ sequence, pSV2 Δ gptMOv18V_H was cut with BamH I and dephosphorylated with alkaline phosphatase (Step 2 in Fig. 6). The human ϵ gene, pUC ϵ [32], was cut with BamH I to release a 2.8-kb fragment containing C ϵ 1–C ϵ 4 (step 3 in Fig. 6) and was ligated into the BamH I site of pSV2 Δ gptMOv18V_H (Step 4 in Fig. 6). The chimeric SF25-IgE [33] was con-

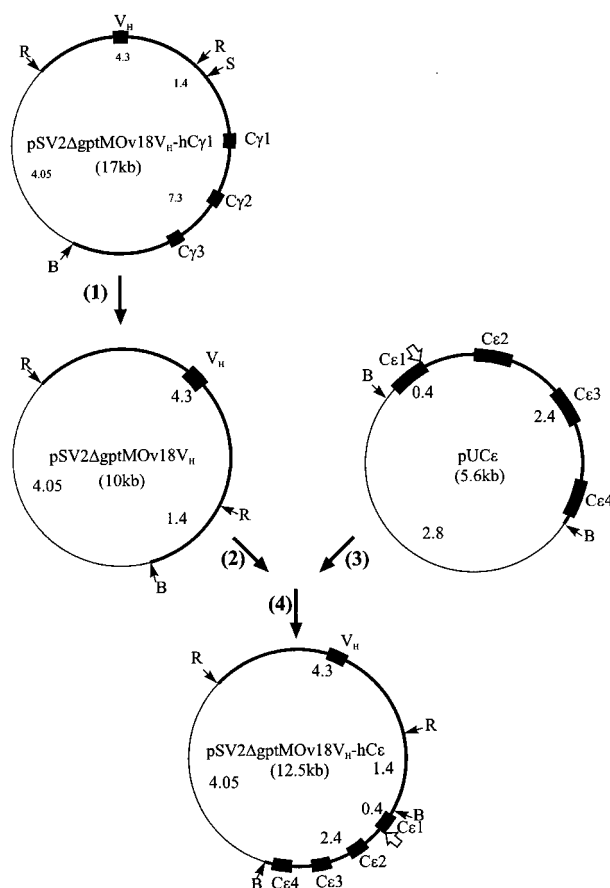


Figure 6. Construction of the MOv18-IgE expression vector: The original materials, the chimeric (murine V_H and human γ 1) expression vector (pSV2 Δ gptV_H-hC γ 1) and the human ϵ -gene in pUC8, and the steps (1–4) involved in recombination to construct the chimeric (murine V_H and human ϵ) expression vector (pSV2 Δ gptMOv18V_H-hC ϵ) are described in detail in Sect. 4.1. Restriction sites (R = EcoR I; B = BamH I, S = Sal I) are indicated (thin arrows) outside the circles and the length of DNA between the sites inside the circles. A diagnostic Sal I site within the ϵ gene is also indicated (fat arrow).

structed in exactly the same manner. The chimeric MOv18- κ light chain vector, containing the V κ regions of MOv18 antibody with the G418 resistance gene (neo), which was constructed according to the same principles, has been described previously [20]. The SF-25 κ light chain vector was constructed in the same manner.

Plasmid DNA was purified by centrifugation through two sequential cesium chloride gradients. Purified DNA was introduced into SP2/0 cells by electroporation. The SP2/0-AG4 (ATCC no. CRL1581) cells were seeded at a density of 2×10^5 – 3×10^5 cells/ml and allowed to grow overnight. Cells (1×10^7 – 2×10^7) were harvested by centrifugation and resuspended in 0.8 ml HBSS without Mg

and Ca (Hazelton Laboratories Inc.). A mixture containing 12 µg each of the κ - and ϵ chain expression vectors in 100 µl glass-distilled water was mixed with the SP2/0 cells in a 0.4-cm electrode gap gene pulser cuvette (BioRad, Richmond, CA). The mixture was pulsed at 200 V in a BioRad Gene Pulser electroporation apparatus and diluted to 1×10^5 cells/ml. This cell suspension was plated in 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA) at a density of 1×10^4 cells/well. Drug for selection was added to a final concentration of 0.25 µg/ml mycophenolic acid, 1.25 µg/ml hypoxanthine, 25 µg/ml xanthine, after 48 h. Colonies resistant to the drug selection were scored, screened for antibody production and expanded after 1–2 weeks. Tissue culture supernatants were screened for chimeric IgE(κ) antibody production by ELISA, and single clones, which generated the highest concentration of IgE, approximately 4 ng/ml for both MOv18-IgE and SF25-IgE, were expanded and used in the present study.

4.2 Protein purification

MOv18-IgE, SF25-IgE and anti-NP IgE from culture supernatants was purified on an immunoaffinity column prepared by coupling anti-IgE mAb 7.12, prepared from a hybridoma line (ATTC HB236), to cyanogen bromide-activated Sepharose (Pharmacia, Piscataway, NJ). The purity of the IgE was assessed by electrophoresis on denaturing 7–25 % gradient polyacrylamide gels under both reducing and non-reducing conditions [43] and by HPLC (Fig. 1). The MOv18-IgG1 was prepared and purified as previously described [20].

4.3 Cell binding assays

MOv18- or SF25-IgE were radiolabeled with ^{125}I , as previously described [34]. To estimate the affinity of MOv18-IgE for the antigen or IGROV1 cells, the cells were grown to confluence and harvested by treatment with 0.25 % trypsin and EDTA. The detached cells were resuspended in RPMI medium (Sigma, St. Louis, MO) containing 2 % FCS and allowed to stand at room temperature for 3 h. After this time, cells were centrifuged and resuspended in RPMI medium with 10 % FCS at 1×10^5 cells/ml. A dilution series of unlabeled IgE, ranging from 0–60 µg/ml, was mixed with a fixed concentration of ^{125}I -labeled IgE (specific activity 6.3×10^5 cpm/µg) at 5.5 µg/ml. Aliquots of the cell suspension (1×10^5 cells) were added, and the samples were incubated on a rotary mixer for 1 h at room temperature. At the end of the incubation the samples were transferred into 0.4-ml microfuge tubes and the cells were sedimented through a phthalate oil mixture consisting of dibutyl phthalate and doctyl phthalate (60:40) and the supernatants removed. The tips of the tubes were cut off and the γ -emission of the cell pellet counted.

The number of Fc ϵ R1 receptors on the IGROV1 cells was measured by the binding of the labeled MOv18-IgE with an increasing number of IGROV1 cells. The kinetics of binding of MOv18- and SF25-IgE were measured by the binding of the labeled antibodies to a CHO cell line expressing Fc ϵ R1, as previously described [34].

4.4 Basophil experiments

Mixed leukocytes containing basophils were prepared by dextran sedimentation from blood and endogenous IgE eluted using lactic acid to increase availability of Fc ϵ R1 [44]. Following passive sensitization with antigen-specific IgE or IgG1 (all at 1 µg/ml), basophils were stimulated either with anti-human IgE (Dako) or a variable number of IGROV1 cells and the reaction allowed to proceed for 45 min. Samples were centrifuged to separate liberated histamine from that remaining in the cells and histamine was quantified using an automated fluorometric assay and expressed as a percentage of the total cellular histamine content. All values were corrected for the spontaneous release of histamine which occurred in the absence of any secretory stimulus (typically around 3 %).

4.5 Platelet experiments

Platelets were isolated as previously described [45]. IgE-rich sera from patients with schistosomiasis or with allergy to house dust mites were selected at the Medical School of the University of Lille. Anti-Fc ϵ R1 mouse mAb 15-1 (mouse IgG1), which is specific for the extracellular region of the Fc ϵ R1 α chain and competitive with IgE, was a gift from Dr. J.-P. Kinet [36]. Subfragments of mAb 15-1 were prepared by ficin to produce (Fab')₂ fragments, which were reduced by 2-ME to give Fab fragments [46]. Sheep anti-human IgE Ab was purchased from Miles Laboratory (Elkart, IN). The efficiency of the digestion was confirmed by gel electrophoresis. Schistosomules were prepared according to previously described techniques [47]. Freshly released *Schistosoma mansoni* cercariae were collected from parasitized snails and transformed into schistosomules by allowing them to penetrate isolated mouse skin. Schistosomules were stored at 37 °C in MEM until use.

For cytotoxicity studies, 50–80 schistosomules in 50 µl EMEM were added to the wells of a 96-well flat-bottom plate containing a 50 µl platelet suspension (1.5×10^8 platelets), together with either normal human serum (negative control, 10 %), the IgE-rich serum specified (positive control, 10 %), MOv18-IgE or SF25-IgE at a final concentration of 15 µg/ml. The final volume was adjusted to 200 µl with Eagle's MEM. Cross-linking of IgE on the surface of platelets was achieved by adding sheep anti-human IgE Ab (Miles Laboratories, Elkart, IN) at 10 µg/ml or 1×10^5 IGROV1 cells. For positive controls, the allergic serum cross-linking of IgE was achieved with specific allergen (*Dermatophagoides pteroi-*

nyssinus, Laboratoire des Allergènes, Fresne, France, 10 µg/ml). The plates were incubated at 37 °C for 24 h and the number of dead schistosomules in each well counted.

To detect the IgE involved in the killing of schistosomules, inhibition experiments were performed with increasing concentrations of anti-FcεRI mAb 15-1 (Fab')₂ fragments, which were added 30 min prior to the MOv18-IgE and IGROV1 cells. As a mouse mAb isotypic control, anti-*Toxoplasma* mouse mAb was used instead of anti-FcεRI.

4.6 ADCC assays

Monolayers containing 0.5×10^6 IGROV1 or LS-180 cells were labeled with 50 µCi ⁵¹Cr for 12 h. The cells were trypsinized and 5×10^3 cells were added to microwells. MOv18-IgE, SF25-IgE or MOv18-IgG1 were tested at 1–15 µg/ml. Blood mononuclear cells were separated on Ficoll, and adherent cells removed during a 2-h incubation on plastic. Lymphocytes were then added to target cells at a ratio of 50:1. Cell lysis was assayed at 4–16 h and results were expressed as % isotope release. Spontaneous release was measured with target cells incubated in medium alone. Similar results were obtained with mononuclear cells, which were not treated to remove adherent cells (not shown).

4.7 SCID mouse experiments

PBMC were prepared by standard techniques from Ficoll-separated whole venous blood and resuspended in PBS at 1.2×10^7 cells/ml for injection into mice. Male C.B-17 scid/scid (SCID) mice (8–10 weeks of age, purchased from Taconic, Germantown, NY) were used for serial passage of the IGROV1 tumor (3×10^6 – 5×10^6 cells s.c.). Tumor masses were extracted, the cells released by disruption in a Foley strainer, suspended in PBS with 0.5 % BSA, and washed 3X before use in experimental tumor challenge. SCID mice were pretreated (–1 day) with rabbit anti-asialo G_{M1} Ab (25 µg i.p.; WAKO Pure Chemical, Richmond, VA), and challenged (day 0) s.c. in the flank with IGROV1 tumor cells (1×10^6 – 3×10^6). Human PBMC (3×10^6 , 0.25 ml PBS) were then injected i.v. into appropriate groups, along with 0.2 ml of either MOv18-IgE (50–100 µg), MOv18-IgG1 (100 µg), or SF-25-IgE (100 µg) Ab. Tumors were measured with calipers at various times after challenge. Tumors that were palpable, but too small to measure accurately, were given a base size of 9 mm², whereas 0 was assigned to mice with no detectable tumors. Statistical analysis was performed by Tukey multiple analysis of variance using Systat 5.2 software.

4.8 Histology

Tumors were excised from the mice during postmortem examination and fixed in formol saline for 24 h. Tissues were

embedded in paraffin before cutting 4-µm sections onto glass microscope slides. For image analysis and histologic observation, all tumor sections were stained with hematoxylin and eosin. For image analysis, microscopic fields of 35 mm² each were randomly selected and enhanced by pseudocolor to emphasize boundaries between viable tumor cells. Necrotic zones were then selected according to spectral differences from viable regions using image analysis, software, NIH Image, Version 1.55, utilizing a computer-coupled Olympus BX50 microscope, equipped with a Sony videocamera. This permitted image analysis software to easily detect differences between these two regions, as has been previously reported [48]. Mapped zones of necrosis were represented as % necrosis/each 0.37 mm² field, with a total field area of 3.7 mm², evaluated for each experimental condition.

Acknowledgements: This work was supported by a Cancer Research Campaign Project Grant (SP1570) and by Centocor, Inc. (HJG) and the United States Public Health Service Grant [CA-60630 (RK)]. We thank Dr. T. H. Rabbitts (MRC Laboratory of Molecular Biology, Cambridge) for providing the cloned ε chain gene, Prof. M. I. Colnaghi and Dr. S. Canevari (Istituto Nazionale Tumori, Milan) for providing MOv18-IgG1, Dr. J.-P. Kinet and Dr. M.-H. Jouvain (Harvard Medical School, Boston) for mAb 15-1 and the CHO cell line expressing FcεRI, Dr. L. M. Lichtenstein (Johns Hopkins Medical School, Baltimore) for the protocol used in the dissociation of IgE from basophils, Prof. F. L. Pearce (University College London) and Prof. M. Greaves (St. John's Institute of Dermatology, St. Thomas' Hospital London) for use of histamine auto-analyzers, and Mr. H. Vong (Institute Pasteur de Lille) for assistance with the platelet studies.

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