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IgE-Antibody-Dependent Immunotherapy of Solid Tumors: Cytotoxic and Phagocytic Mechanisms of Eradication of Ovarian Cancer Cells

Sophia N. Karagiannis,1* Marguerite G. Bracher,* James Hunt,* Natalie McCloskey,* Rebecca L. Beavil,* Andrew J. Beavil,* David J. Fear,* Richard G. Thompson, † Nicholas East, † Frances Burke, † Robert J. Moore, † David D. Dombrowicz, ‡ Frances R. Balkwill, † and Hannah J. Gould*

Abs have a paramount place in the treatment of certain, mainly lymphoid, malignancies, although tumors of nonhemopoietic origin have proved more refractory ones. We have previously shown that the efficacy of immunotherapy of solid tumors, in particular ovarian carcinoma, may be improved by the use of IgE Abs in place of the conventional IgG. An IgE Ab (MOv18 IgE) against an ovarian-tumor-specific Ag (folate binding protein), in combination with human PBMC, introduced into ovarian cancer xenograft-bearing mice, greatly exceeded the analogous IgG1 in promoting survival. In this study, we analyzed the mechanisms by which MOv18 IgE may exert its antitumor activities. Monocytes were essential IgE receptor-expressing effector cells that mediated the enhanced survival of tumor-bearing mice by MOv18 IgE and human PBMC. Monocytes mediated MOv18 IgE-dependent ovarian tumor cell killing in vitro by two distinct pathways, cytotoxicity and phagocytosis, acting respectively through the IgE receptors FcRI and CD23. We also show that human eosinophils were potent effector cells in MOv18 IgE Ab-dependent ovarian tumor cell cytotoxicity in vitro. These results demonstrate that IgE Abs can engage cell surface IgE receptors and activate effector cells against ovarian tumor cells. Our findings offer a framework for an improved immunotherapeutic strategy for combating solid tumors. The Journal of Immunology, 2007, 179: 2832–2843.

Ovarian cancer ranks second among gynecological cancers in the number of new cases and first in the number of deaths each year (1, 2). In the early stages, it grows in and around the ovaries and it metastasizes in the peritoneal cavity and later to the internal organs. It causes no symptoms in the early stages, and is essentially incurable in the late stages of the disease; around 50–60% of patients die within 5 years (1, 2). Ovarian cancer is intractable to current chemotherapies, but immunotherapies using IgG Abs currently in clinical trials are showing some promising results (3–6).

The current use of the IgG1 Ab isotype in Ab immunotherapy stems from Waldmann’s classic work (7, 8) on the efficacy of Campath-1H (alemuzumab) IgG Ab subclasses in complement-dependent immunotherapy of non-Hodgkin’s lymphoma. Several other mechanisms are now known to operate in Ab immunotherapies (3, 9). However, non-IgG isotypes have never been tried in cancer patients. IgG Abs are now increasingly being used for the treatment of blood malignancies, but “solid” tumors are often refractory (3–5). Probable reasons are diffusion barrier of tissues, slow recruitment of effector cells, and low affinity of IgG for its receptors (8–10).

Abs of the IgE class may offer an alternative to the conventional treatments with IgG Abs, particularly for solid tumors such as those of the ovary. Unlike Abs of the IgG class, IgE binds to its receptors with very high affinity. The affinity of an IgE Ab for its high affinity receptor, FcεRI (Kₐ = 10¹¹ M⁻¹), is two to five orders of magnitude higher than that of IgG for the FcγRs (FcγRIII) (9–11). Its affinity for the low affinity receptor, CD23 (Kₐ = 10⁸ M⁻¹), is as high as that of IgG for FcεRII (11). Furthermore, the peritoneal cavity, where ovarian cancer spreads, is home to IgE receptor-expressing cells, such as macrophages (12, 13), mast cells (14), and dendritic cells (15). These properties may translate to strong retention of Abs in tissues and longer antitumor immune surveillance. IgE may therefore have greater efficacy than IgG in targeting tumors in nonhemopoietic tissues.

A number of epidemiological studies on the relation between allergies and the risk of cancer support the idea that IgE might have advantages over IgG for the treatment of certain types of cancer. Mills et al. (16) conducted a prospective study in 34,198 Seventh-Day Adventists. They found that the risk of prostate, breast cancer, and lymphatic or hematopoetic cancers actually increased with allergy, and further increased with an increasing number of allergies. However, the risk of ovarian cancer was decreased with allergy and decreased with an increasing number of...
allergies. A recent meta-analysis has demonstrated a significant inverse association between a history of both asthma and hay fever and overall cancer mortality and colorectal cancer (17). Recent studies have also confirmed the increased risk of prostate and breast cancer (18), and shown an increased risk for lung cancer with asthma and a decreased risk of pancreatic cancer and glioma (19–24).

Experimental studies have also supported the concept of harnessing IgE for cancer therapy. Nagy et al. (25) demonstrated that a mouse monoclonal IgE, directed against the murine mammary tumor virus, prevented the growth of the tumor in mice. Kershaw et al. (26) showed that a mouse monoclonal IgE, directed against a human colon carcinoma Ag, conferred a brief survival advantage to mice implanted with colon tumor cells. We have shown that a chimeric Ab, MoV18 IgE, directed against an ovarian tumor Ag, folate binding protein, in combination with human PBMC, was more active than MoV18 IgG1 in protection of mice from ovarian tumor growth in two xenograft models of ovarian carcinoma in scid and nude mice (27, 28).

T cells do not express FcεRI, and hence treatment with IgE Abs would not exploit their known cytotoxic functions. Kershaw et al. (29) expressed the extracellular portion of the high-affinity IgE receptor, FcεRI α-chain, joined to the membrane sequence of mouse FcγRII and cytoplasmic sequences of CD28 and the TCR ζ-chain (CD28-ζ) in primary human T cells (30). FcεRI-CD28-ζ T cells, together with an IgE Ab directed against CD8, protected scid mice from the growth of a human thymoma tumor. This strategy should allow any tumor Ag-specific IgE or combination of IgEs to be used in adoptive cell immunotherapy of any type of cancer.

Another way of exploiting the inherent advantages of IgE over IgG is based on oral vaccination of mice with tumor Ags under alkaline conditions, which favors the production of IgE Abs (31, 32). Jensen-Jarolim et al. (33) showed that oral vaccination with a breast tumor “Ag” stimulates the production of IgE Abs that activate IgE effector cells and mediate tumor cell lysis in vivo. Reali et al. (34) have shown that passive immunization with IgE Abs can also result in stimulating an active immune response. This reflects the sensitization of APCs bearing FcεRI, which results in uniquely strong stimulation. It is notable that no adverse effects of IgE Ab treatment in the mouse models of cancer have been observed in any of the above-mentioned studies.

The present study focuses for the first time on the mechanism of IgE-dependent tumor cell killing. For this, we made use of MoV18 IgE developed against the tumor-associated Ag folate binding protein (35, 36), which is overexpressed in 80% of ovarian cancers (37, 38). In a previous study, we observed that monocytes infiltrated human ovarian tumors growing in the nude mice treated with MoV18 IgE and human PBMC (28). In the present study, we show that monocytes are necessary for the protection of the mice by human PBMC. Human monocytes express the two IgE receptors, the high-affinity receptor, FcεRI, and the low-affinity receptor, CD23 (37–41). CD23 expression is induced by IL-4 on a wide range of hemopoietic cells, including monocytes (42, 43), and has been shown to act in IgE Ab-dependent phagocytosis (ADCP) of hapten-coated red cells (44). The nearest IgG receptor homologue to FcεRI is FcγRIII, which acts in IgG Ab-dependent T cell-, NK cell-, and macrophage-mediated tumor cell cytotoxicity (Ab-dependent cell-mediated cytotoxicity, ADCC) (9, 10, 45). In this study, we use a novel three-color cytometric assay (46) to enable us to distinguish between two modes of IgE-dependent monocyte-mediated tumor cell killing, cytotoxic cell killing (ADCC), and phagocytosis (ADCP), and establish that FcεRI is responsible for ADCC and CD23 for ADCP.

Eosinophils express low levels of FcεRI, which mediates IgE-dependent stimulation of IL-10 secretion and defense against parasites (47). Using purified eosinophils from blood, we now show that they, too, are potent effector cells in MoV18 IgE Ab-dependent ovarian tumor cell killing in vitro.

Materials and Methods

Abs and reagents

Chimeric Abs MoV18 IgE against folate binding protein and 4-hydroxy-3-nitro-phenacetyl (NIP) IgE specific for the hapten NIP were prepared as described (27, 48). We used goat anti-human IgE-FITC Ab (Vector Laboratories), anti-CD89-FITC (BD Biosciences), anti-CD14-PE, isotype control mAbs, and anti-mouse IgG (Fab)2-FITC (DakoCytomation). Anti-CD23 mAbs IDEC-152 and IDEC-152 Fab (Dr. J. Hopp, Biogen Idec, San Diego, CA) recognize the IgE binding site (49). Anti-FcεRI mAb 22E7 recognizes an epitope unaffected by IgE occupancy (Hoffmann-La Roche) (50). The soluble FcεRI α-chain (sFcεRIα) was prepared as before (51). Human IL-4 (1U = 34.5 pg) was obtained from R&D Systems. Propidium iodide (PI), CFSE dye, tissue culture medium, and reagents were obtained from Invitrogen Life Technologies.

Flow cytometric evaluation of receptor expression and IgE binding to monocytes

Monocytes were incubated with 10 μg/ml mAb 22E7 or MM6, anti-mouse IgG (Fab)2–FITC, followed by anti-CD14-PE. To assess IgE binding, monocytes were given 5 μg/ml MoV18 IgE or no Ab for 30 min at 4°C, followed by 10 μg/ml goat anti-IgE-FITC for 30 min at 4°C. To block IgE binding to cell surface receptors, 5 μg/ml MoV18 IgE were incubated alone or with 62 μg/ml sFcεRIα for 30 min at 37°C, followed by addition of monocytes and anti-IgE-FITC. Incubations and washing steps were performed in FACS buffer (PBS, 5% normal goat serum).

Cell purification, stimulation, and culture

The human ovarian carcinoma IGROV1 cells were grown in RPMI 1640, 10% FCS complete medium at 37°C in 5% CO2 (52). Monocytes from human venous blood were isolated to 70–80% purity as described before (27, 28). Cells were incubated overnight at 37°C in AIM-V medium, 5% FCS, in VルーLife (FEP) culture bags (American Fluoroseal Corporation) (53). Monocytes were incubated for 20 h with 320 U/ml (10 ng ml–1) human rIL-4 to stimulate CD23 or 2 μg/ml MoV18 IgE to stimulate FcεRI. For in vivo experiments, PBMCs were isolated from human venous blood as before (27, 28). PBMCs were depleted of monocytes by incubation with CD14-mAbs and CFSE dye to label monocytes, followed by the removal of labeled monocytes using a VarioMACS immunomagnetic device (Miltenyi Biotech) according to the manufacturer’s instructions. Eosinophils were isolated to >95% purity by Percoll gradient centrifugation (density 1.082 g/ml) (GE Healthcare) followed by immunomagnetic separation with anti-CD16-coated immunomagnetic beads as previously described (47), and used for assays immediately. All work was performed with the approval of the Guy’s Research Ethics Committee and with the volunteers’ written informed consent.

Flow cytometric cytotoxicity/phagocytosis (ADCC/ADCP) assay

Cell treatment. A three-color flow cytometric assay was used to simultaneously study tumor cell cytotoxicity (ADCC) and phagocytosis (ADCP) of IGROV1 cells by human effector cells as previously described (46). IGROV1 cells were labeled with 10–24 mm CFSE for 10 min at 37°C 1 day before assays. A total of 1.3 × 106 CFSE-labeled IGROV1 cells were mixed with 1.3 × 105 unstained effectors (E/T ratio = 1:1) and 5 μg/ml MOV18, NIP IgE, or no Ab, followed by incubation for 2.5 h at 37°C. In blocking experiments, 25 μg/ml IDEC-152 Fab were added to monocytes for 30 min at 37°C before assays. In others, 62 μg/ml sFcεRIα were combined with 5 μg/ml IgE Abs or with complete medium alone for 30 min at 37°C, followed by addition of cells. All conditions were tested in triplicate. Cells were then incubated with 10 μg/ml anti-CD89-FITC mAb to label monocytes or anti-CD49d-FITC to label eosinophils for 25 min at 4°C, washed, and treated with 0.25 μg/ml PI for 15 min at 4°C to identify dead cells. Following
a further wash, cells were mixed thoroughly to interrupt cell-cell contact, and 20,000 cellular events were acquired by flow cytometry using a dual laser FACScanLibur flow cytometer (BD Biosciences).

**Assay setup and calculations.** Acquisition and measurement of single cell events were monitored by forward scatter vs side scatter dot plots and compared with control single- and mixed-population samples. CFSE-labeled IGROV1 were detected in FL1 (530/30 nm band pass filter), PE-labeled effectors in FL2 (582/42 nm band pass filter), and PI+ dead cells in FL3 (670 nm LP band pass filter) channels. Appropriate controls were set for compensation adjustments between fluorochromes and ADCC and ADCP (46, 54). To calculate ADCC and ADCP (Fig. 1), two dot plots were generated and three regions were identified: 1) R1 (green), total CFSE+ tumor cell targets. Region 2 (R2, orange), CFSE+/PE- cells = tumor cells phagocytosed by PE+ effector cells; and 3) R3 (red), CFSE+/PI- cells = intact dead tumor cells. Deviations between samples were accounted for by “R1 Spontaneous Loss (SL) Control” = the average R1 of three control samples (i.e., effector and target cells without mAb).

Below are calculations to determine the proportion of IGROV1 tumor cells killed by ADCC and ADCP:

- **R1 SL control – R1 = X**
- **ADCC = [(X + R3)/R1 SL Control] × 100**
- **ADCP = [(R2/R1 SL Control) × 100**

**Immunofluorescence imaging of cells**

Monocytes were incubated on glass chamber slides (SLS) with IGROV1 and mAbs to assess contact between cells and ADCP as previously described (28, 46). Following incubations, monocytes were given anti-CD89-PE mAb. In similar assays, anti-CD94-PE mAb was used to label eosinophils. Slides were washed, fixed in 1% paraformaldehyde-FACS buffer, and mounted with fluorescence preserver (DakoCytomation). Slides were observed using an Axioskop 20 upright microscope (Carl Zeiss) equipped with a Zeiss A-Plan 40X/0.65 Ph2 lens, an AxioCam 14-bit camera, and AxioVision Version 3.0.2 imaging system (Imaging Associates). Light and fluorescent images were superimposed as described before (28).

**Experiments in the human ovarian carcinoma xenograft model**

The human ovarian carcinoma xenograft HUA was established in 8–12-wk-old specific-pathogen-free female nude mice and implanted i.p. as described before (28). Monocytes depleted of monocytes by effector cells. Dot plots of mixed monocyte effectors and IGROV1 tumor target cells from which calculations were made. Region 1 (R1, green) represents total CFSE+ tumor cell targets. Region 2 (R2, orange) depicts the CFSE+ tumor cells present within PE-stained monocytes (CFSE+/PE-), depicting phagocytosis. Region 3 (R3, red) contains tumor cells killed externally by effector cells (cytotoxicity) and are therefore CFSE+/PI-.

**Results**

**CD23 expression on monocytes and role in IgE-mediated ADCP of tumor cells**

In previous studies, we used standard cytotoxicity assays to examine the efficacy of tumor Ag-specific IgE for the immunotherapy of ovarian cancer (27, 28). Standard cytotoxicity assays do not measure phagocytosis. Thus, they may underestimate tumor cell killing and hence the potential of an Ab for immunotherapy of cancer. To analyze the mechanisms by which IgE effector cells mediate tumor cell killing, we have developed a three-color cytometric assay (Fig. 1) to simultaneously measure cytotoxicity and phagocytosis of tumor cells by effector cells and tumor Ag-specific IgE (46).

Two-color flow cytometric dot plots of CD14+ human monocytes show that <3% of monocytes cultured without IL-4 stimulation express CD23 (Fig. 2A, top). After overnight stimulation with IL-4, 58% of the cells express CD23. The proportion of monocytes expressing FceRI (31%) remained unchanged after IL-4 stimulation (Fig. 2A, middle). Thus, incubation of monocytes with IL-4 stimulated expression of CD23, but did not affect expression of FceRI on the cell surface. With IL-4 stimulation, the proportion of monocytes capable of binding IgE increased from 32% in untreated monocytes to 56% in IL-4-treated cells (Fig. 2A, bottom), suggesting that newly expressed CD23 on the surface of monocytes was capable of binding MOv18 IgE.

We measured ADCC and ADCP of IGROV1 cells cultured for 2.5 h with human peripheral blood monocytes and either MOv18 IgE, the hapten-specific anti-IgE, or no Ab as controls (Table I: n = 6). Unstimulated monocytes mediated 34.4% ADCC, compared with <15% for the NIP IgE and the no Ab controls (Fig. 2B, Table I). No phagocytosis of tumor cells was detected by comparison to controls. Following IL-4 stimulation of the monocytes, MOv18 IgE ADCP was 32.5%, compared with <10% for the controls, whereas MOv18 IgE-mediated ADCP measured at 22.2%, compared with <15% for the control samples.

Thus, IL-4 stimulation did not affect MOv18 IgE-mediated ADCP (p = 0.77, n = 6), but it significantly enhanced MOv18 IgE ADCP (p = 0.0007, n = 6). Levels of ADCP in control samples without Ab or with NIP IgE were also slightly elevated compared with those for unstimulated monocytes (Table I). This suggests that IL-4 may enhance the innate phagocytic capacity of monocytes.

Because IL-4 stimulates the expression of CD23, but not FceRI (Fig. 2A), the results of the three-color assay imply that ADCP
FIGURE 2. CD23 up-regulation by IL-4 and role in MOv18 IgE-mediated ADCP of ovarian tumor cells. A, Two-color flow cytometric dot plots show IgE receptor expression and binding of MOv18 IgE in primary monocytes. Primary monocytes, untreated (left) or incubated with IL-4 (right), were labeled with anti-CD23 mAb IDEC-152 (top) or anti-FcεRI mAb 22E7 (middle), followed by anti-mouse IgG (Fab)2–FITC (x-axis) and CD14-PE (y-axis). For binding of MOv18 IgE to untreated (bottom left) and IL-4-stimulated monocytes (bottom right) cells were labeled with MOv18 IgE, anti-IgE FITC (x-axis), followed by CD14-PE (y-axis). B, MOv18 IgE-mediated killing of IGROV1 tumor cells by untreated (left) and IL-4-stimulated (right) monocytes. C, ADCP killing of tumor cells by IL-4-stimulated primary monocytes (left) was blocked by anti-CD23 IDEC-152 mAb Fab (right). Cytotoxicity (ADCC), black bars; phagocytosis (ADCP), gray bars. Results shown are means ± SD of six independent experiments. Significance of values compared with samples given MOv18 IgE by the Student’s t test. n/s, p > 0.05; *, p < 0.05; **, p < 0.005; ***, p < 0.0005.
may be attributed to CD23. To test this possibility, we preincu-
ated monocytes with 20-fold molar excess of the IDEC-152 
CD23-blocking Ab Fab (Fig. 2C; n = 6). Following IL-4 stimu-
lation of monocytes, MOv18 IgE-induced ADCC was 26.2%, 
compared with <15% for the controls. As before (Fig. 2B), with 
IL-4 stimulation, MOv18 IgE-induced ADCP measured at 25.8%, 
compared with <15% with the controls. Blocking of IgE binding 
to CD23 with IDEC-152 Fab resulted in MOv18 IgE ADCP mea-
sured at 26.1%, compared with <15% for the controls. Monocytes 
treated with the CD23 blocking Ab Fab, however, showed low 
MOv18 IgE ADCP (13.9%), a value similar to that measured with 
the controls.

### Table I. Blocking of IgE-mediated killing of IGROV1 tumor cells by monocytes

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<tr>
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<th>% ADCP ± SD</th>
<th>% ADCC ± SD</th>
<th>% ADCP ± SD</th>
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<td>Untreated Monocytes (n = 6)</td>
<td>IL-4-Treated Monocytes (n = 6)</td>
<td>Untreated Monocytes (n = 6)</td>
<td>IL-4-Treated Monocytes + sFcRIOAb (n = 6)</td>
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<td>9.9 ± 1.2**</td>
<td>8.1 ± 3.0***</td>
<td>9.7 ± 3.1***</td>
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<tr>
<td>NIP IgE</td>
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<td>7.7 ± 3.5***</td>
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<td>14.2 ± 4.3*</td>
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<td>14.7 ± 4.9**</td>
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<td>26.2 ± 8.7</td>
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<td>5.4 ± 1.1</td>
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<td>MOv18 IgE</td>
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<td>12.0 ± 1.6</td>
<td>16.5 ± 4.2</td>
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* Monocytes were incubated with or without human rIL-4 to stimulate CD23 expression. IGROV1 tumor cells were incubated with monocyte effector cells and MOv18 IgE, NIP IgE, or no Ab, followed by incubation for 2.5 h at 37°C. To block CD23, IDEC-152 Fab was added to monocytes prior to assays. To block binding of IgE to receptors, sFcRIOAb was combined with IgE before addition of cells. Significance compared to samples given MOv18 IgE by the Student’s t test: n/s, p > 0.05; *, p < 0.05; **, p < 0.005; ***, p < 0.0005.

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**FIGURE 3.** FcRI is responsible for monocyte ADCC by tumor Ag-specific IgE. A. Two-color flow cytometric dot plots show FcRI expression in primary monocytes. Monocytes were incubated in medium alone (left) or stimulated with MOv18 IgE for 20 h (middle). Cells were labeled with anti-FcRI mAb 22E7, anti-mouse IgG (Fab)2–FITC (y-axis), followed by anti-CD14–PE (x-axis). The proportion of monocytes showing cell surface FcRI expression increased from 31.0 ± 9.6% to 37.8 ± 10.1% following overnight stimulation with IgE (n = 8). B. Increased FcRI cell surface expression correlated with enhanced ADCC of IGROV1 tumor cells by primary monocytes and MOv18 IgE. Primary monocytes were incubated overnight in medium alone or stimulated with medium containing MOv18 IgE, followed by ADCC/ADCP tumor killing assays. Monocytes from eight donors were assessed, and each set of ADCC measurements represents mean values of triplicate calculations. Tumor cell killing by IgE-stimulated monocytes and MOv18 IgE increased to 30.7 ± 8.1% compared with 24.4 ± 7.7% with unstimulated monocytes and MOv18 IgE (n = 8). Phagocytosis was not significantly influenced by IgE stimulation (ADCP: 8.1 ± 3.2% with and 6.4 ± 1.4% without IgE, n = 8, p > 0.05). Significance of changes in IgE receptor expression and in tumor cell killing by the paired two-tailed Student’s t test: **, p < 0.005; ***, p < 0.0005.
Whereas MOv18 IgE ADCC remained unchanged at 26% with or without CD23 blocking (p = 0.97), MOv18 IgE ADCP was reduced from 25.8% to 13.9% with blocking of CD23 (p = 0.006). Thus, CD23 mediated the IgE ADCP of ovarian tumor cells effected by monocytes. The observed levels of expression of FcεRI and CD23 in unstimulated and IL-4-stimulated monocytes (Fig. 2A), together with the activity of anti-CD23, suggest that FcεRI mediates the constitutive ADCC and CD23 mediates the superimposed ADCP in IL-4-stimulated monocytes (Fig. 2, B and C).

**Up-regulation of FcεRI on monocytes and function in IgE ADCP of tumor cells**

We established that monocyte-mediated ADCP is due to the action of CD23 by correlating the increase in ADCP with the up-regulation of CD23 by IL-4, and by the inhibitory effect of the anti-CD23-blocking Ab. Similarly, we have been able to attribute ADCC to the action of FcεRI. We up-regulated FcεRI by IL-4-stimulated monocytes (Fig. 3A), together with the activity of anti-CD23, suggest that FcεRI mediates the constitutive ADCC and CD23 mediates the superimposed ADCP in IL-4-stimulated monocytes (Fig. 2, B and C).

Primary monocytes were incubated for 20 h with or without MOv18 IgE, and then labeled with CD14-PE and the 22E7 anti-FcεRI Ab to determine the level of receptor expression by flow cytometry. Preincubation with MOv18 IgE led to a modest increase, in the proportion of FcεRI⁺-expressing CD14⁺ cells (Fig. 3A, representative dot plots). Without stimulation, 31.0% of CD14⁺ cells expressed FcεRI and this proportion increased to 37.8% of the cells in IgE-stimulated monocytes (Fig. 3A, right; n = 8). Increased expression of FcεRI on monocytes stimulated with MOv18 IgE in culture corresponded to an equivalently modest increase in MOv18 IgE-induced ADCP from 24.4% in unstimulated monocytes to 30.7% in IgE-stimulated monocytes (Fig. 3B; n = 8). ADCP was measured at background levels in unstimulated and IgE-stimulated monocytes. These results support a role for FcεRI in IgE-mediated ADCP.

IgE bound to IL-4-stimulated monocytes was assayed using goat anti-IgE-FITC. We detected endogenous IgE bound to 25% by the Student’s t test: n/s, p > 0.05; *, p < 0.05; **, p < 0.005; ***, p < 0.0005.

![Figure 4](http://www.jimmunol.org/)
proves that ADCC depends on MOv18 IgE binding to FcεRI on monocytes. In the case of IL-4-treated monocytes, sFcεRIα led to decreases in both MOv18 IgE ADCC (from 27.8% to 12.0%; \(p = 0.002, n = 6\)) and MOv18 IgE ADCP (from 16.5% to 5.8%; \(p = 0.0001, n = 6\)), approaching the values observed in controls (Fig. 4C). This was expected because, as mentioned above, sFcεRIα blocks the binding of IgE to both receptors (11, 56, 57). We conclude that the binding of MOv18 IgE to both IgE receptors on monocytes contributed to IgE-mediated tumor cell targeting and killing.

Visualization of contact between tumor cells and monocytes

To visualize the results, shown quantitatively in the flow cytometric assays described above, we labeled IGROV1 with CFSE before incubation with IgE Abs for 2.5 h and then stained monocytes with anti-CD89-PE. Cell interactions were observed by fluorescence microscopy. Incubation with MOv18 IgE led to contact between monocytes and IGROV1 cells (Fig. 5A), not seen with control Ab NIP IgE, or when the MOv18 IgE was preincubated with 100-fold molar excess of sFcεRIα (Fig. 5A). When the monocytes were stimulated by IL-4, contact between the tumor and effector cells was enhanced, and phagocytosis was clearly visible in the merged image of the green tumor cells inside the red monocytes, resulting in the yellow color (Fig. 5B; arrow); this is not seen with NIP IgE, or when IgE binding to its receptors is blocked by sFcεRIα. Addition of the IDEC-152 anti-CD23 blocking mAb Fab to the monocytes cultured with IL-4 substantially reduced, but did not eliminate, the contact between monocytes and IGROV1 cells. IDEC-152 Fab inhibited phagocytosis, seen by the absence of yellow color inside the monocytes (Fig. 5B). In contrast, incubation with sFcεRIα completely inhibited contact between monocytes and IGROV1 cells, consistent with IgE binding to both FcεRI and CD23 and the inhibition of cytotoxicity as well as phagocytosis (Fig. 4, B and C).
Monocytes are necessary for IgE-mediated survival of mice bearing ovarian tumor xenografts

We tested the ability of monocytes to promote survival in nude mice bearing the human ovarian carcinoma xenograft HUA, which expresses the folic acid receptor at moderate levels (28). HUA cells were derived from a patient with ovarian cancer; they have not yet been adapted to tissue culture and their characteristics have not changed in years of passage in mice. The tumors grow and spread in the peritoneal cavity of the mice, as in ovarian cancer patients, and their histological and cytokine profiles are similar to the original ascites. This is, therefore, considered a more clinically relevant model than the ovarian carcinoma cell line IGROV1 used in this study (27, 28, 55). HUA ascites were introduced into nude mice by i.p. injection. Unlike human IgG1, the Fc fragment of the human IgE (FcεRI) is not recognized by the murine FcεRI (10). Thus, to study the effect of human IgE and the role of IgE receptor-bearing effector cells in targeting a human ovarian carcinoma xenograft, we introduced human mononuclear cells from peripheral blood. Mice were treated with human effector cells, with or without MOv18 IgE, and the length of survival was assessed (Fig. 6).

Mice treated with PBMCs plus MOv18 IgE survived for 39 days and they had a significant survival advantage compared with mice that received PBMC alone (21 days) or buffer controls (16 days) (Table II). As observed in our previous studies (27, 28), PBMCs alone survived longer than buffer controls. Mice given PBMCs depleted of monocytes plus MOv18 IgE survived for 21 days, while those treated with PBMCs depleted of monocytes without IgE survived for 18 days and their survival was shorter than that of mice given unfractionated PBMC with MOv18 IgE. Removal of monocytes from the PBMC population thus abolished the survival advantage of mice given PBMC and MOv18 IgE, implying that monocytes play a crucial role in targeting IgE against tumor cells and promoting mouse survival.

The role of monocytes in delaying tumor growth and prolonging mouse survival was further confirmed with reconstitution of monocyte-depleted PBMCs with purified monocytes. Adding back purified monocytes to depleted PBMCs at proportions equivalent to those in unfractionated PBMCs restored the ability of PBMCs and MOv18 IgE to increase mouse survival (44 days) to levels statistically equivalent to those seen in mice given whole PBMCs and MOv18 IgE (Fig. 6, Table II). This survival was significantly longer than monocyte-reconstituted PBMCs alone (25 days) or depleted PBMCs with and without MOv18 IgE. Purified monocytes

Monocytes are crucial effector cells in IgE-mediated survival of ovarian carcinoma xenograft-bearing mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (n = 21)</td>
<td>15.8 ± 2.7***</td>
</tr>
<tr>
<td>PBMC (n = 21)</td>
<td>20.6 ± 5.1**</td>
</tr>
<tr>
<td>PBMC + MOv18 IgE (n = 21)</td>
<td>38.7 ± 23.9</td>
</tr>
<tr>
<td>Monocyte-depleted PBMC (n = 13)</td>
<td>18.4 ± 3.0**</td>
</tr>
<tr>
<td>Monocyte-depleted PBMC + MOv18 IgE (n = 13)</td>
<td>20.5 ± 5.8*</td>
</tr>
<tr>
<td>Monocyte-depleted PBMC + Monocytes (n = 13)</td>
<td>24.9 ± 9.4*</td>
</tr>
<tr>
<td>Monocyte-depleted PBMC + Monocytes + MOv18 IgE (n = 13)</td>
<td>44.4 ± 16.4n/s</td>
</tr>
</tbody>
</table>

* Results are mean ± SD. Numbers in brackets denote the number of nude mice examined in each group. Significance levels for comparison to mice treated with PBMC and MOv18 IgE by the Student’s t-test: n/s, p > 0.05; *, p < 0.05; **, p < 0.005; *** p < 0.0005.
were also tested in this model, but they did not confer any survival advantage to tumor-bearing mice (results not shown). This may be due to the requirement of survival factors or trafficking signals conferred by other cells in the PBMC preparation in these models.

Eosinophils mediate IgE-dependent ADCC

Eosinophils were lost in the purification of PBMCs from blood. To test their ability to direct IgE Ab-dependent tumor cell killing, we purified eosinophils and used them as effector cells in the ADCC/ADCP assay. We measured the ADCC and ADCP of IGROV1 cells incubated with human eosinophils (red) were incubated with IgE Abs for 2.5 h. After incubation with MOv18 IgE, eosinophils were in frequent contact with IGROV1 cells and IGROV1 tumor cell destruction was evident (left). Eosinophil-IGROV1 cell contact was observed less frequently in samples given control NIP IgE (middle), or no Ab (right). Original magnification, ×400. Scale bars, 20 μm.

Table III. IgE-mediated killing of IGROV1 tumor cells by eosinophils

<table>
<thead>
<tr>
<th>Fresh Eosinophils (n = 4)</th>
<th>% ADCC ± SD</th>
<th>% ADCP ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Ab</td>
<td>14.6 ± 5.4*</td>
<td>6.6 ± 3.7ns</td>
</tr>
<tr>
<td>NIP IgE</td>
<td>16.7 ± 5.2*</td>
<td>6.8 ± 3.9ns</td>
</tr>
<tr>
<td>MOv18 IgE</td>
<td>32.4 ± 8.6</td>
<td>5.9 ± 3.1</td>
</tr>
</tbody>
</table>

* Human eosinophils freshly isolated from peripheral blood were incubated with IGROV1 tumor cells and MOv18 IgE, NIP IgE or no Ab for 2.5 h at 37°C. Significance compared to samples given MOv18 IgE by the Student’s t test: n/s, p > 0.05; *, p < 0.05.

Discussion

Following our previous demonstration that MOv18 IgE is superior to MOv18 IgG1, together with human PBMC, in prolonging the survival of mice in our xenograft models of ovarian cancer (27, 28), we sought to identify the IgE effector cells and the IgE receptors and mechanisms involved in tumor cell killing. The observation of monocytes infiltrating the xenografts in the mice (28) directed our attention to monocytes. We also examined the activity of eosinophils, which are potential IgE effector cells not present in PBMC.

In earlier work, we observed that FcεRI, but not CD23, was constitutively expressed on primary monocytes, implicating the former receptor in IGROV1 cell killing (28). In this study, we confirmed this finding and found that FcεRI-expressing primary monocytes exerted primarily ADCC. Upon up-regulation of FcεRI by preincubation of the monocytes with MOv18 IgE, we observed
an increase in ADCC, further suggesting that FcεRI mediates cytotoxic cell killing. ADCC and the contact between the monocytes and tumor cells, observed by fluorescence microscopy, were inhibited by sFcεRIα, confirming this mechanism of FcεRI action. In contrast, up-regulation of CD23 by IL-4, led to MOv18 IgE ADCP without affecting the level of ADCC. Moreover, incubation with the IDEC-152 anti-CD23 Ab Fab inhibited ADCP, but not ADCC. Therefore, we were able to attribute IgE-mediated cytotoxicity of tumor cells to FcεRI and IgE-mediated phagocytosis to CD23.

Our tumor cell killing assays and microscopic observations suggest that contact of tumor target and monocytes is necessary for IgE-mediated ADCC and ADCP. Addition of tumor Ag-specific IgE was needed for tumor cell death, as the hapten-specific NIP IgE did not mediate tumor killing. This implies the requirement for tumor cell recognition by a tumor Ag-specific Ab. Blocking of MOv18 IgE binding to receptors on monocytes by preincubation of IgE with sFcεRIα drastically reduced MOv18-IgE-mediated ADCC and ADCP. Therefore, engagement of IgE receptors by IgE is also required. Finally, microscopic observations show a correlation between E:T cell contact and the presence of MOv18 IgE. Therefore, bridging tumor cells and IgE receptors on monocytes by IgE is necessary for both mechanisms of IgE-mediated tumor killing, ADCC and ADCP, to occur.

Our observations in human ovarian carcinoma xenograft-bearing mice clearly suggest a pivotal role for monocytes in enhancing mouse survival effected by MOv18 IgE. In this in vivo system, monocytes in PBMC, together with MOv18 IgE, are required to enhance mouse survival, compared with controls. This implies that IgE-receptor-bearing cells like monocytes can act, not only in vitro, but also in vivo as effector cells. Monocytes-macrophages are recruited in the intratumoral environment by tumor-derived chemotactic signals. Tumor-associated macrophages can be activated to promote tumor growth in situ and fail to mount an antitumor response (13, 53, 58). Resident macrophages express IgE receptors (59) and are capable of mediating NO release (60). Based on our observations on the IgE-mediated antitumor effects of monocytes in vitro as well as in vivo, we propose that these cells may be activated and reprogrammed by a tumor Ag-specific IgE to kill and phagocytose tumor cells, rather than promote their growth.

The PBMC used in our xenograft model contain around 2% human basophils (28), which are known to express FcεRI. We have shown that these cells can be activated to secrete histamine in the presence of PBMC, tumor cells, and MOv18 IgE (27). It is likely that IgE-dependent mast cell and basophil activation by tumor cell Ags would enhance the recruitment of inflammatory cells to the site of a tumor, but this remains to be tested.

The PBMC used as effector cells lack other IgE receptor-expressing cells, such as eosinophils, tissue mast cells, and macrophages that might mediate strong antitumor responses. The absence of these potent human effector cells in this system may have contributed to the observed limited efficacy that MOv18 IgE confers in this system. We have previously observed a lack of enhanced mouse survival using tumor-specific IgE to treat established tumors; these observations could also be attributed to lack of potent human effector cells. Human PBMC have a limited lifespan in the mouse and their antitumor effects may therefore be limited to a short time following treatments. Cytokines released by human effector cells may not act on murine effector cells and, thus, the xenograft model may not reveal indirect mechanisms of tumor cell killing. Our results may point toward a beneficial role for IgE therapy following surgical intervention on ovarian cancer patients with smaller tumor burden or with minimal residual disease.

IgE-FcεRI complexes on eosinophils are known to exert ADCC against parasites (61, 62). In this study, we show for the first time that a tumor Ag-specific Ab can direct eosinophils to kill tumor cells by ADCC. As observed with monocytes, and consistent with the expression of low levels of FcεRI on the surface of eosinophils (47), this IgE receptor on eosinophils appears to mediate MOv18-dependent cytotoxicity of tumor cells. Engagement of IgE Abs on the surface of eosinophils triggers the release of inflammatory and cytotoxic mediators, such as eosinophil peroxidase and major basic protein (63), which may contribute to tumor cell death. Our data, therefore, raise the possibility that eosinophils, either resident in intratumoral areas or recruited from the circulation, could contribute to the elimination of tumor cells in vivo. Indeed, eosinophil infiltrates are associated with host inflammatory responses against a number of cancers (62, 64).

The human immune system operates with nine Ab classes, but cancer immunotherapy has been attempted only with Abs of the IgG class. It seems unlikely that this would afford optimal efficacy for all types of tumors, in every anatomical location and in every cancer patient. Other workers have shown IgA ADCD and ADCP of breast tumor cells (54, 65), melanoma cells (66), and non-Hodgkins lymphoma (67) by monocyte-derived macrophages (65) or neutrophils (54, 66, 67). IgA Abs were directed to tumor cells in the form of bispecific Abs against the IgA receptor. FcεRI or CD89, on the effector cells and the specific tumor Ag on the tumor cells. Bispecific Abs were used to compensate for the low affinity of IgA for FcεRI (≈10\(^{-6}\) M\(^{-1}\)), which precludes the stable interaction of Ab with the effector cells. Unlike either IgG or IgA, IgE binds to FcεRI with sufficiently high affinity (≈10\(^{-11}\) M\(^{-1}\)) to be effective as a monospecific Ab. Consistent with the stability of the IgE-FcεRI interaction, circulating monocytes and eosinophils are able to transport IgE into tissues where this IgE may engage effector cells in tumor surveillance and killing, as we have shown (11, 13, 59, 61, 62). This property is also likely to enhance Ag presentation by FcεRI APCs (e.g., dendritic cells) leading to active immunity.

There is an increasing call for rational therapies for the treatment of cancer, requiring knowledge of mechanisms of drug action (68). There are no previous reports that analyze the mechanisms of IgE-dependent tumor cell killing and the role of IgE receptor-expressing effector cells. Our findings offer a framework for an improved immunotherapeutic strategy for combating solid tumors.

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Disclosures
The authors have no financial conflict of interest.

References
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