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Immunology

Interleukin 3 enhances cytotoxic T lymphocyte development and class I major histocompatibility complex "re-presentation" of exogenous antigen by tumor-infiltrating antigen-presenting cells

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ABSTRACT **We show that interleukin 3 (IL-3) enhances the generation of tumor-specific cytotoxic T lymphocytes (CTLs) through the stimulation of host antigen-presenting cells (APCs). The BALB/c (H-2^d) spontaneous lung carcinoma line 1 was modified by gene transfection to express ovalbumin as a nominal "tumor antigen" and to secrete IL-3, a cytokine enhancing myeloid development. IL-3-transfected tumor cells are less tumorigenic than the parental cell line, and tumor-infiltrating lymphocytes isolated from these tumors contain increased numbers of tumor-specific CTLs. By using B3Z86/90.14 (B3Z), a unique T-cell hybridoma system restricted to ovalbumin/H-2^b and implanting the tumors in (BALB/c × C57BL/6)F$_1$ (H-2$^{d/b}$) mice, we demonstrate that the IL-3-transfected tumors contain an increased number of a rare population of host cells that can process and "re-present" tumor antigen to CTLs. Electron microscopy allowed direct visualization of these host APCs, and these studies, along with surface marker phenotyping, indicate that these APCs are macrophage-like. The identification of these cells and their enhancement by IL-3 offers a new opportunity for tumor immunotherapy.**

Cytotoxic T lymphocytes (CTLs) recognize target cells through their class I major histocompatibility complex (MHC) molecules, which contain peptide generally derived from endogenous or cytoplasmic sources (1, 2). The generation of CTLs also appears to require costimulatory molecules such as B7 found on professional antigen-presenting cells (APCs) (3, 4). Because tumor cells generally lack potent costimulatory molecules, tumor cells are likely to be very inefficient at stimulating CTLs. Therefore, generation of the tumor-reactive CTL response may require uptake of exogenous tumor antigen, processing, and "re-presentation" by professional APCs. The phenomenon of re-presentation or cross-priming was described many years ago in several systems including simian virus 40 (5) and minor histocompatibility antigens (6, 7), but the mechanism and the cells responsible have remained elusive. These cells would have importance for antigen processing and presentation with class I antigens and also be of practical importance for the design of tumor vaccines. If tumor antigens can be ingested and efficiently processed by such APCs, tumor cells used in vaccines would only have to carry the tumor antigen and would not require matching to the patient's HLA type. Similarly, purified antigens could be delivered in such a way as to be efficiently re-presented by these host cells. Interleukin 3 (IL-3) is a cytokine known to stimulate hematopoietic precursors, particularly of the myeloid lineage (8). Our earlier observations that IL-3 dramatically increased the development of CD8$^+$ anti-tumor CTLs and that these CTLs were essential for the cytokine-enhanced tumor rejection (9) led us to hypothesize that IL-3 was enhancing CTL development by increasing re-presentation. Here we show that IL-3 increases the CTLs generated due to re-presentation and also increases the number of macrophage-like APCs capable of re-presentation within the tumors.

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MATERIALS AND METHODS

Mice. (BALB/cByJ × C57BL/6J)F$_1$ (H-2$^{d/b}$) mice were purchased from The Jackson Laboratory and used at 2–4 months of age.

Plasmids. I-356, the plasmid containing class I H-2K^b cDNA (10, 11), and the pEVRF0-PvuII/XbaI-Ova (489) plasmid, which contains a truncated form of ovalbumin (Ova; ref. 12), were subcloned into pHβ-Apr-1-neo and the pHβ-Apr-1-gpt vectors, respectively (13), using PCR to incorporate appropriate (*Sal* I, *Hin*dIII, and *Bam*HI) restriction sites. The *Hin*dIII/*Xba* I fragment of pcDNA1/neo-Ova plasmid, which contains the full-length Ova cDNA (12, 14), was subcloned into the corresponding sites in pUC19 to create a *Bam*HI site at the 3′ end of the Ova cDNA. The *Hin*dIII/*Bam*HI fragment was then subcloned into the corresponding sites in the pHβ-Apr-1-neo and pHβ-Apr-1-gpt vectors.

Cell Lines and Transfectants. Line 1 is a H-2^d lung carcinoma derived from a BALB/c mouse (15). EL4 is a H-2^b T-cell thymoma derived from a C57BL/6 mouse (16). B3Z86/90.14 (B3Z) is a somatic T-cell hybrid generated by fusing the Ova/K^b-specific cytotoxic clone, B3, with a *lacZ*-inducible derivative of the BW5147 fusion partner (17). Line 1/IL-3 (9) or parental line 1 cells were transfected by using the human β-actin expression vectors encoding either the full-length Ova or truncated Ova-(138–386) (Ova^{ns}) or K^b cDNAs using Lipofectin (GIBCO/BRL) according to the manufacturer's instructions. EL4 cells were transfected by electroporation. Selection with the appropriate drug was performed as described (18), after which cells were cloned by limiting dilution and screened by ELISA for Ova expression or by flow cytometry for class I expression.

Monoclonal Antibodies and Host Cell Depletions. The monoclonal antibodies used in these studies were Y-3, anti-(H-2K^b) class I MHC (19); AF4-62.4, anti-(H-2D^d) class I MHC (20); M5-114, anti-class II (21); T24/40.7, anti-Thy-1 (22); GK1.5, anti-CD4 (23); 2.43, anti-CD8 (23); 14.8, anti-B220 (24); 33D1 and NLDC, anti-DC markers (25); F4/80, anti-macrophage (26); 2.4G2, anti-Fcγ receptor (27); M1/70,

Abbreviations: CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; APC, antigen-presenting cell; IL-3, interleukin 3; B3Z, B3Z86/90.14; Ova, ovalbumin; Ova^{ns}, nonsecreted form of Ova [Ova-(138–386)]; TIL, tumor-infiltrating lymphocyte; EM, electron micrograph; DC, dendritic cell; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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anti-(Mac-1) (28); 30-F11, anti-CD45 (22); J11d, anti-heat stable antigen (HsAg) (29); YN1/1.4.7, anti-ICAM-1 (30); 1G10, anti-B7-1 (31). These antibodies were coated onto 2×10^7 sheep anti-rat IgG paramagnetic beads (Dynabeads M-450) and incubated with 4×10^7 cells from tumor-infiltrating lymphocyte (TIL)-depleted tumor suspensions on a rotator for 45 min at 4°C; magnetic bead-coated cells were removed using a Dynal magnet. To test if the cells were adherent, adherent cells were depleted by plating 10^7 cells in 60-mm Petri dishes for 2 hr at 37°C. The tumor suspensions remaining after each type of depletion were renormalized by plating 1.5×10^6 cells per well and were cocultured with 5×10^5 B3Z cells as described below.

ELISA, TIL, and Antigen Presentation Assays. Ova in culture supernatants collected from 2×10^5 cells growing for 48 hr in 2 ml of medium or in cell lysate from 10^8 cells was assayed using an antibody-sandwich ELISA with minor modifications (32). Thy-1^+ cells were purified as described (18) using sheep anti-rat IgG paramagnetic beads (Dynabeads M-450; Dynal) conjugated with T24/40.7 (anti-Thy-1) from tumors growing i.m. in the thighs of (BALB/c × C57BL/6)F_1 mice 20 days after injection of 10^5 tumor cells. Cytolytic activity against ^{51}Cr-labeled target cells (2000 per well) was determined as described (9). For antigen presentation assays with B3Z cells, 4×10^5 cells of the indicated cell lines or serially diluted tumor suspensions depleted of TIL were cocultured with 2×10^5 or 5×10^5 B3Z cells, respectively, for 16 hr in 24-well plates. Fixation and 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) substrate incubation was performed as described (17).

Electron Microscopy (EM). B3Z cells for transmission EM analysis were either not activated or activated with anti-CD3 (Boehringer Mannheim) as described (33). The host re-presenting APCs from line 1/Ova/IL-3 tumor were enriched by adherence to plastic and were cocultured with 10^6 B3Z cells for 16 hr in 35-mm Petri dishes. Cultures were fixed with 2% EM-grade glutaraldehyde for 40 min on ice and were incubated with X-Gal as described (17). Postfixation through embedment in Polybed 812 was performed in the culture dishes. Sections (40–80 nm) were placed on Formvar-coated slotted grids and were photographed unstained in a Zeiss 10CR transmission electron microscope.

RESULTS AND DISCUSSION

Development of Re-Presentation System. We wished to develop an experimental model that would enable us to follow a tumor antigen as it traveled from the tumor cell to a host APC. To accomplish this, it was first necessary to create a model tumor antigen since in the line 1 mouse lung carcinoma (15), as in many tumor systems, the tumor antigen is unknown. A cDNA encoding Ova was transfected into the line 1 cells. We also took advantage of mouse genetics to enable us to specifically measure both APCs and CTLs that arise solely as a result of re-presentation (see Fig. 1). This was monitored experimentally by injecting transfected line 1 cells (H-2^d) expressing either Ova alone (line 1/Ova) or Ova plus IL-3 (line 1/Ova/IL-3 or line 1/Ova^{ns}/IL-3) into (BALB/cByJ × C57BL/6J)F_1 mice (H-$2^{d/b}$) (Fig. 1 and Table 1). By measuring cytotoxic activity specific for Ova in the H-2^b haplotype, we ensured that the analyzed CTLs resulted solely from re-presentation. Using the same experimental protocol, we exploited a β-galactosidase reporter T-cell hybridoma (B3Z), which recognizes the K^b class I molecule and an Ova peptide, SL8 (SIINFEKL) (12), to characterize the APCs within the tumor that had re-presented Ova.

IL-3 Enhances CTL Derived by Re-Presentation. We measured the Ova/H-2^b-restricted cytotoxic activity of the TILs to examine the CTL response that arises solely from host APC re-presentation *in vivo*. TILs isolated from the line 1/Ova/IL-3

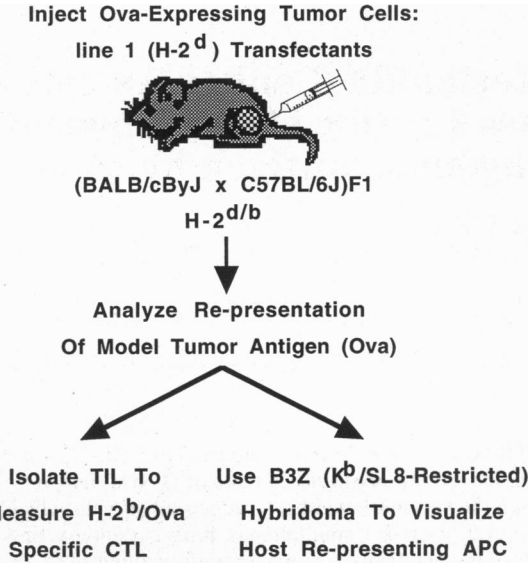

FIG. 1. Experimental design used to measure exogenous antigen re-presentation in the context of class I MHC. (BALB/cByJ × C57BL/6J)F_1 (H-$2^{d/b}$) hybrid mice at age 2–4 months were injected i.m. with 10^5 cells of the H-2^d line 1/Ova transfectant or the H-2^d line 1/Ova/IL-3 or the H-2^d line 1/Ova^{ns}/IL-3 double transfectants.

tumor lysed H-2^b target cells substantially better than TILs isolated from line 1/Ova tumor (Fig. 2*A*). For example, at a 100:1 ratio (effector to target), EL4/Ova targets were lysed significantly (≈35%) by line 1/Ova/IL-3 TIL, whereas line 1/Ova TIL did not lyse these targets above background levels.

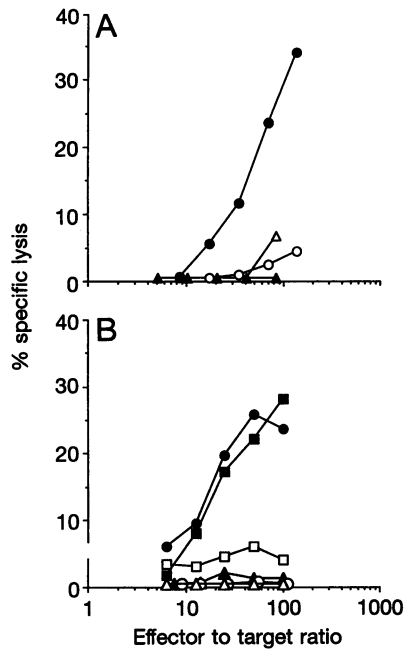

FIG. 2. Analysis of TIL from line 1 tumors. (*A*) (BALB/cByJ × C57BL/6J)F_1 (H-$2^{d/b}$) mice were injected i.m. with 10^5 line 1/Ova/IL-3 cells expressing secreted Ova (circles) or line 1/Ova cells (triangles). TILs were harvested using paramagnetic beads on day 20, pooled from two mice according to the tumor types, and assayed for their cytolytic activity on ^{51}Cr-labeled H-2^b target cells EL-4 (open symbols) and EL-4/Ova (closed symbols). (*B*) TILs were harvested from line 1/Ova/IL-3 expressing secreted Ova (circles), line 1/Ova^{ns}/IL-3 expressing the nonsecreted form of Ova (squares), or line 1/Ova (triangles) as described above and assayed on EL-4 (open symbols) or EL-4/Ova (closed symbols). Spontaneous release was <10% of the maximal release for both targets.

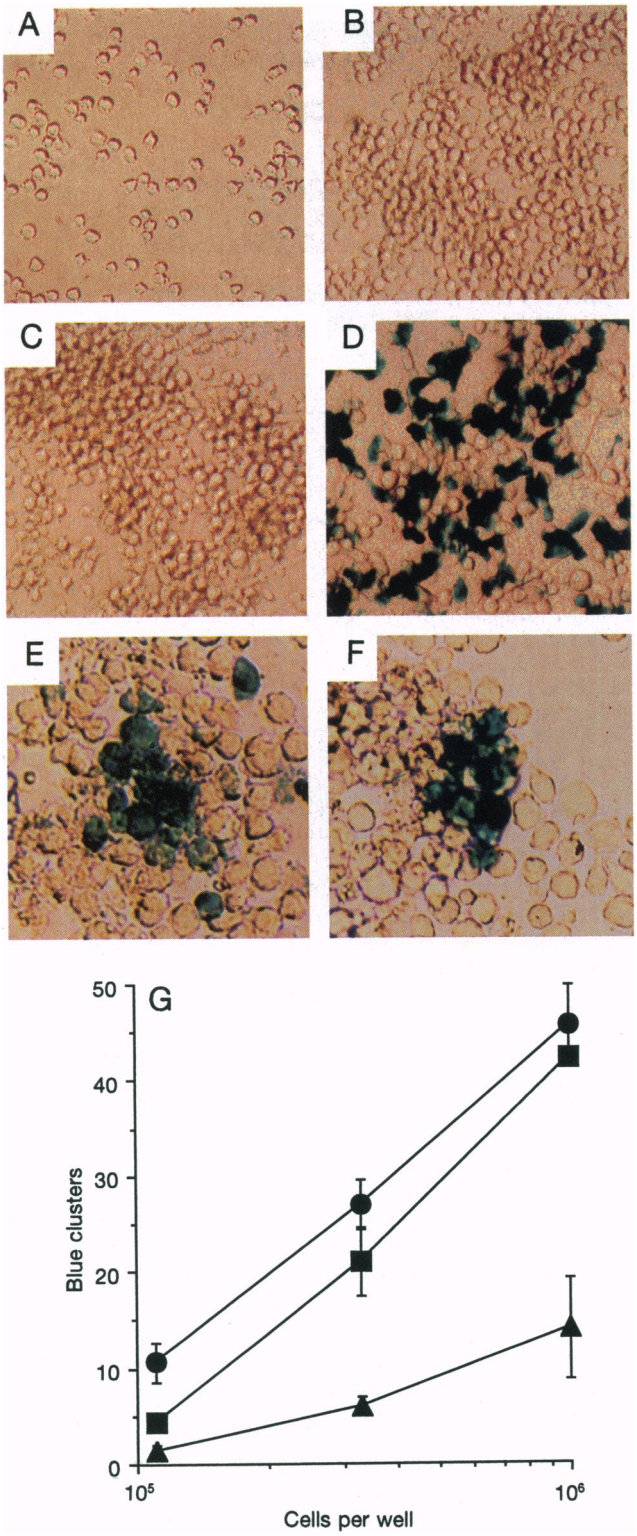

FIG. 3. Analysis of antigen re-presenting cells. (*A–D*) B3Z T-cell hybridoma responds only to cells that coexpress K^b class I MHC and Ova. B3Z were cultured alone (*A*) or in the presence of line 1 cells transfected with K^b (line 1/K^b) (*B*), line 1/Ova cells (*C*), or line 1 cells transfected with both Ova and K^b (line 1/Ova/K^b) (*D*). (*E* and *F*) Representative example of activated B3Z clusters resulting from coculture of B3Z cells with line 1/Ova/IL-3 tumor (*E*) and with line 1/Ova^{ns}/IL-3 tumor (*F*). (*G*) Line 1/Ova/IL-3 and line 1/Ova^{ns}/IL-3 tumors contain an increased number of host APCs re-presenting Ova compared to line 1/Ova tumor. Serially diluted line 1/Ova/IL-3 (●), line 1/Ova^{ns}/IL-3 (■), or line 1/Ova (▲) tumor suspensions isolated from tumors 14 days after implantation were cocultured with B3Z to measure the amount of host cell re-presentation of exogenous Ova with class I MHC. Data represent the number of blue (activated) B3Z clusters per well, with each cluster scored as one. Note that each cluster contained at least three blue B3Z cells. In the case of the tumor suspensions, each point was done in quadruplicate. (*A–D*, × 100; *E* and *F*, × 400.)

Table 1. Characteristics of transfected cell lines

Cell line*	IL-3,† pg/ml	Secreted Ova,† ng/ml	Internal Ova,‡ ng/ml
Line 1	0	0	0
Line 1/Ova	0	41	100
Line 1/Ova/IL-3	1000	31	100
Line 1/Ova^{ns}/IL-3	1000	0	100

*Line 1 cells or line 1/IL-3-secreting cells (previously transfected with IL-3 using the neo selectable marker) were transfected with either secreted or nonsecreted Ova cDNA expression vectors using the gpt marker. Clones expressing similar amounts of secreted Ova were selected and used in these experiments.

†Amount of IL-3 in pg/ml or Ova in ng/ml produced when 2×10^5 cells were plated in 2 ml of medium for 48 hr.

‡Amount of Ova in ng/ml analyzed by ELISA assays performed on Triton X-100 detergent cell lysates (cells lysed at a concentration of 10^8 cells per ml).

Data with EL4 pulsed with the SL8 peptide gave similar, although lower, lysis patterns (data not shown). The absolute yield of TILs in this experiment was 1.2 times higher from the line 1/Ova/IL-3 tumor than from the line 1/Ova tumor. Similar results were obtained in three independent experiments. Since these cell lines express similar amounts of Ova (see Table 1), these results show that IL-3 enhances CTL development by host cell re-presentation.

To examine the effect of secretion of the Ova tumor antigen, the experiments described above were repeated with line 1 tumor cells transfected with a truncated form of Ova that lacks a leader sequence so it is not secreted (Table 1; ref. 14). As shown in Fig. 2*B*, the TILs recovered from tumors containing IL-3 and this nonsecreted form of Ova have cytotoxic activity strikingly similar to that obtained from tumors that secrete Ova. In addition, the total yield of TILs from the nonsecreting tumors was very similar to that from the tumors secreting Ova. It is significant that our previous work showed that IL-3 reduced tumorigenicity of line 1 cells and *in vivo* depletion studies showed that $CD8^+$ cells were essential for tumor rejection (9). Here we have shown that re-presentation, which could play a critical role in the generation of tumor-reactive CTLs, is enhanced by IL-3.

IL-3 Enhances Antigen Re-Presentation Within the Tumor. The experiments described above showed that the generation of CTLs was the result of antigen re-presentation. To examine antigen presentation directly, we analyzed the number of host cells within the tumor capable of re-presenting Ova using the B3Z hybridoma system. Because B3Z cells recognize SL8/K^b, only host cells re-presenting Ova are detected in these experiments. This is illustrated by control experiments shown in Fig. 3 *A–D*, confirming that the B3Z hybridoma is specific for SL8/K^b. We then analyzed the ability of cells within the tumor to present antigen to B3Z as outlined in Fig. 1. Host cells from the line 1/Ova/IL-3 tumor form characteristic clusters with activated (blue) B3Z cells around a host cell(s) (Fig. 3*E*). Interestingly, the Ova does not appear to need to be secreted, since qualitatively similar clusters were observed when the APCs were derived from line 1/Ova^{ns}/IL-3 tumors, which do not actively secrete Ova (Fig. 3 *E* and *F*). By counting the number of these clusters, each of which must contain at least one host APC, we have quantitated the number of APCs from the line 1/Ova tumors compared to line 1/Ova/IL-3 and line 1/Ova^{ns}/IL-3 tumors. Host cells derived from line 1/Ova/IL-3 or from line 1/Ova^{ns}/IL-3 tumors show many more clusters of

activated B3Z cells than ones isolated from line 1 Ova tumors (Fig. 3*G*). The similar numbers of clusters from line 1/Ova/IL-3 as compared to line 1/Ova^{ns}/IL-3 indicate that active secretion of Ova is not necessary for this effect.

IL-3 Is Necessary for the Enhanced Ova Re-Presentation. Similar amounts of Ova are produced by the line 1/Ova and line 1/Ova/IL-3 cell lines (Table 1), yet the line 1/Ova clone engenders many fewer re-presenting APCs. Further, the secretion of Ova does not seem to enhance the CTL generation or the re-presenting cells. Interestingly, H-2^b cell lines pulsed with whole Ova or with supernatants from line 1/Ova or line 1/Ova/IL-3 do not activate B3Z cells (data not shown). These results make it unlikely that host cells are simply activating B3Z cells in culture by degradation of whole protein and subsequently exchanging free Ova peptides at the cell surface and instead favor a phagocytic model.

It is evident that the line 1/Ova/IL-3 tumors *in vivo* contain an expanded population of host cells that re-present exogenous antigen. From the results in Fig. 3*G*, and assuming that each cluster contains a single APC, the frequency of cells capable of re-presenting exogenous antigen can be estimated. Further assuming that line 1 tumors contain about equal numbers of host and tumor cells (34), $\approx$1 in every 10^4 cells isolated from the line 1/Ova/IL-3 tumor can activate B3Z. The apparent rarity of this cell is not due to our inability to detect the specialized APCs in the tumor mixtures. To test the sensitivity of the B3Z antigen presentation assay, line 1 tumors grown in BALB/c mice were mixed with line 1/Ova/K^b cells, which are capable of directly stimulating B3Z (Fig. 3*D*). Results showed that this assay can detect very low percentages (0.02%) of APCs in complex cell mixtures (data not shown). Thus, as reported previously (17), we believe that it is likely that B3Z can detect most cells expressing significant levels of SL8/K^b even in tumor mixtures.

Phenotype of Cells Re-Presenting Exogenous Antigen. We wished to characterize further the cells within tumors with antigen-presenting activity. Depletion experiments using adherence or monoclonal antibodies to remove cell populations from the tumor mixtures were performed. Subsequent analysis of the remaining cells in the B3Z cell assay was used to analyze the phenotype of the re-presenting cells. Allowing the tumor cell mixture to adhere to plastic for 2 hr depleted >75% of the activated B3Z clusters (Fig. 4). Additional depletion studies were performed using monoclonal antibodies bound to magnetic beads to remove the cells expressing those antigens. The remaining cells were renormalized to a set cell number before performing the B3Z cell assay (Fig. 4). The re-presenting cell was not depleted with antibodies to B220, CD4, or CD8, indicating that the re-presenting cell is not a lymphocyte. Activity was depleted with antibodies to LCA, FcγII receptor, Mac-1, class II, and B7–1, suggesting that this re-presenting cell is a macrophage-like professional APC. The adherence data is also consistent with this finding. F4/80, generally considered a macrophage marker, failed to deplete; however, this marker is not present on all macrophages (35, 36). Discriminating definitively whether the APC is a macrophage or dendritic cell (DC) using cell surface marker analysis is not possible due to the heterogeneity among the cells in the DC family and the overlap in expression of cell surface markers with macrophages. For example, the FcγII receptor is not present on splenic DCs but is on macrophages and Langerhans cells (37). In this regard, the antibodies 33D1 and NLDC, which may be the most specific for DCs, do not deplete the re-presenting cell, suggesting that it is not a classical DC. However, depletion experiments should be interpreted cautiously, since low levels of antigen may not be sufficient to enable the cells to be depleted, and the conditions within the tumor environment may decrease the expression of cell surface antigens. Nevertheless, based on the adherence properties and the antibody depletion data, the re-presenting cells appear macrophage-like.

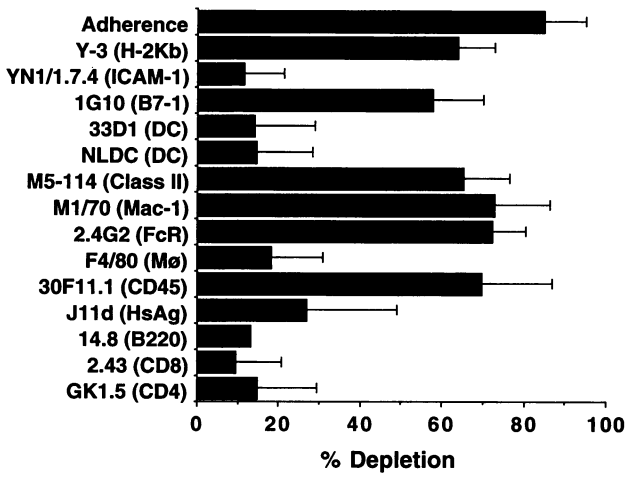

FIG. 4. Characterization of host APCs in the IL-3 tumor. *In vitro* negative selection with antibody-coated magnetic beads or adherence to plastic was used to phenotype the host APCs in the line 1/Ova/IL-3 tumor that had re-presented Ova. All monoclonal antibodies listed are rat IgG2b except B7/BB1 (rat IgG2a), J11d (rat IgM), and Y-3 (mouse IgG2b). The percent depletion was calculated as (1 − average number of clusters in antibody-depleted wells/average number of clusters in the normal rat serum control wells) × 100. The average represents the percent depletion values from three or four individual experiments, except 14.8 (one experiment). All the antibodies were tested for function by flow cytometry and/or depletion using cells expressing the appropriate marker.

Direct Visualization of Re-Presenting Cells. We also examined directly the host cell re-presenting to B3Z by using EM. Transmission EMs of nonstimulated (Fig. 5*A*) versus stimulated (Fig. 5*B*) B3Z illustrate that the activation of β-galactosidase and subsequent hydrolysis of the X-Gal substrate forms an electron-dense marker at the nuclear membrane and endoplasmic reticulum. In essence, this observation allowed us to determine which B3Z cells are activated (i.e., "blue") even at the EM level. Transmission EM analyses was performed on 14 different activated B3Z clusters. In all cases, a non-B3Z cell was present within the cluster, which exhibited a phenotype shown in Fig. 5 *C* and *D*. In many cases, two or more B3Z cells were observed in close contact to a host cell. One such representative cluster is shown in Fig. 5*C*. This APC exhibited numerous vacuoles consistent with it being phagocytic. In addition, it had numerous microvillous projections and an acentric and convoluted nucleus (Fig. 5*D*), morphologic characteristics consistent with it being a macrophage (38, 39).

Re-Presenting APCs and the Generation of CTLs. Our studies have shown that IL-3 dramatically increases the number of re-presenting APCs within the tumors and specific CTLs arising as a result of re-presentation of this tumor antigen. This extends our previous studies showing that IL-3 engenders specific CTLs that were required for tumor rejection (9). The apparently low number of APCs in tumors without IL-3 raises the intriguing possibility that antigen presentation may normally be a rate-limiting step in the generation of tumor-reactive CTLs. The cells capable of re-presenting antigen are relatively rare, consistent with previous observations that exogenous antigen generally does not induce a class I-restricted response. These cells may be rare because (*i*) they are a small subpopulation of macrophages, (*ii*) they are a particular activation state, (*iii*) only a few cells have taken up sufficient exogenous antigen, and (*iv*) they are actively destroyed by mature CTLs as seen in some lymphocytic choriomeningitis virus infections (40). Whatever the reason for their scarcity,

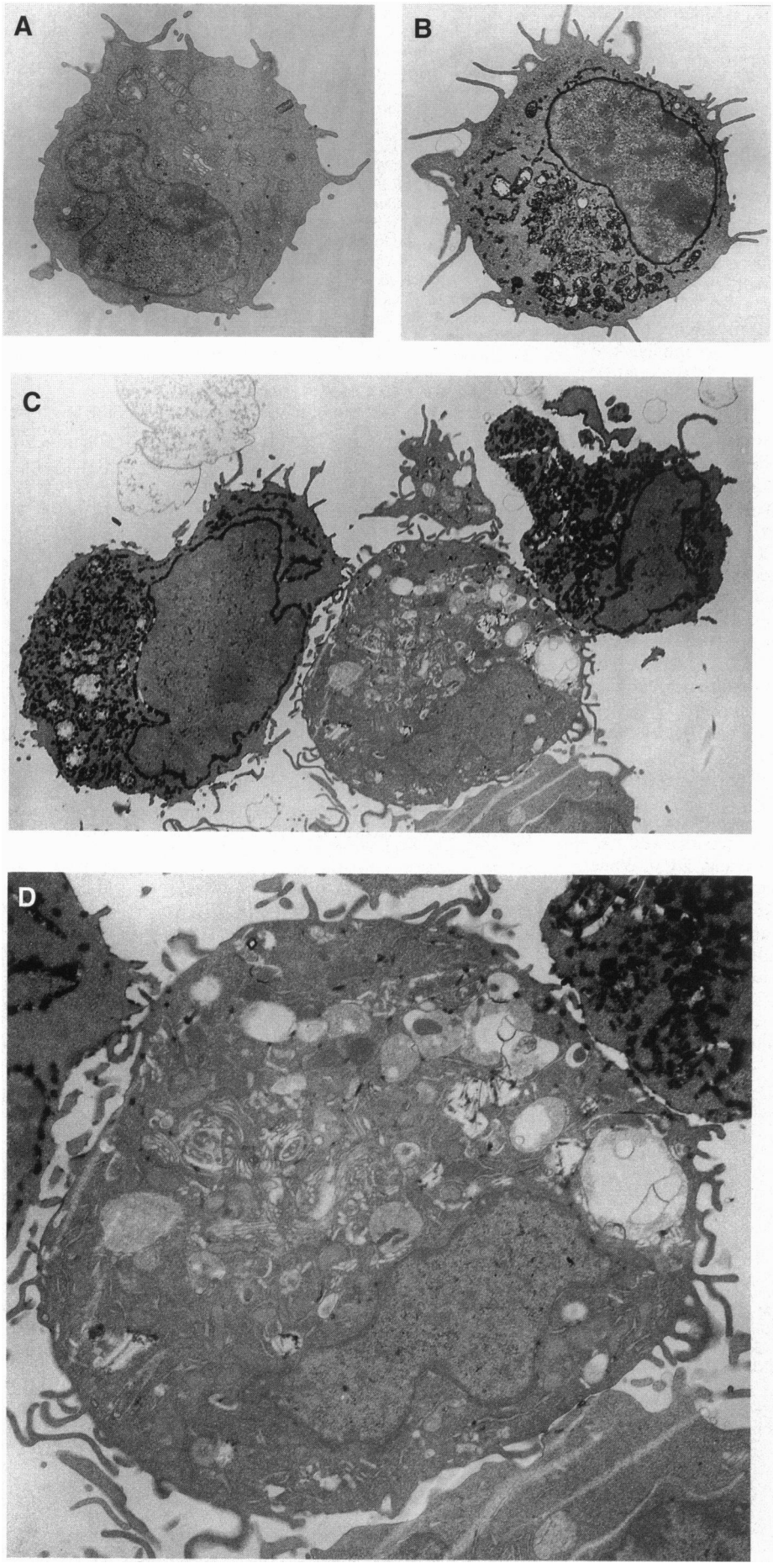

FIG. 5. Transmission electron micrographs of unstained sections of unstimulated B3Z (×4500) (*A*), anti-CD3-activated B3Z in which β-galactosidase hydrolyzed the X-Gal substrate to form an electron dense-marker, which appears blue with light microscopy (×3050) (*B*), macrophage-like APCs interacting with activated (electron dense) B3Z (×3150) (*C*), and macrophage-like APCs at higher magnification (×8625) (*D*).

IL-3 greatly increases these APCs, which is associated with an increase in CTLs.

Characteristics of Cells Responsible for Re-Presentation. Our analysis of the re-presenting APCs revealed cells with several interesting features. These cells induce extensive clustering of T cells (Fig. 3), a trait that these cells share with dendritic cells (41). Here we have also shown that IL-3 enhanced both class I-restricted exogenous antigen representation and generation of primary CTLs stimulated by host-derived APCs. In this regard, classical DCs have been reported to be potent, and perhaps the major, stimulators of primary CTLs against viruses (42). However, this may reflect the ability of many viruses to infect the DCs, where they can provide peptide in the normal class I MHC pathway. Indeed, classical mature DCs do not present exogenous antigen efficiently (37). In contrast, recent reports have shown that macrophages are able to present exogenous particulate antigens in the context of class I molecules (43–46). Relevant to an antitumor response, macrophages could ingest whole tumor cells, cell fragments, or perhaps heat shock proteins complexed to antigenic peptides (47–49) and then process the tumor antigens for presentation. The similar CTL levels generated to Ova, whether it was secreted or nonsecreted, would also favor a phagocytic model of exogenous antigen acquisition. In this regard, we have recently shown that IL-3 treatment of bone marrow generates APCs that can phagocytose exogenous antigen and when transfered to naive recipients, engender a potent primary CTL response (K.-Y.Y. and J.G.F., unpublished observations). Although classical macrophages have generally been considered much poorer APCs than DCs, particularly for primary responses (37), this may reflect a quantitative rather than a qualitative difference. Interestingly, the re-presenting cells that we have characterized by morphology and by cell surface marker analysis appear macrophage-like. These cells could be a specific activation or differentiation state of macrophage or even represent a distinct subpopulation of macrophage. Since IL-3 stimulates myeloid progenitors, an intriguing possibility is that the re-presenting cell we have characterized is a precursor cell that shares characteristics of both macrophages and DCs. While additional experiments are necessary to determine the precise relationship of these re-presenting cells to DCs and macrophages, these cells are likely responsible for the antigen re-presenting functional activity observed in previous studies with simian virus 40 (5) and minor histocompatibility antigens (6, 7) and in more recent chimeric studies (50). Identification of these re-presenting APCs, their enhancement by IL-3, and the subsequent increased generation of antitumor CTLs offers a new avenue for tumor immunotherapy.

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