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Regulation of Human Intestinal Intraepithelial Lymphocyte Cytolytic Function by Biliary Glycoprotein (CD66a)¹


Human small intestinal intraepithelial lymphocytes (iIEL) are a unique population of CD8αβ⁺ TCR-αβ⁺ but CD28⁻ T lymphocytes that may function in intestinal epithelial cell immunosurveillance. In an attempt to define novel cell surface molecules involved in iIEL function, we raised several mAbs against activated iIELs derived from the small intestine that recognized an Ag on activated, but not resting, iIELs. Using expression cloning and binding studies with Fc fusion proteins and transfectants, the cognate Ag of these mAbs was identified as the N domain of biliary glycoprotein (CD66a), a carcinoembryonic Ag-related molecule that contains an immune receptor tyrosine-based inhibitory motif. Functionally, these mAbs inhibited the anti-CD3-directed and lymphokine-activated killer activity of the P815 cell line by iIELs derived from the human small intestine. These studies indicate that the expression of biliary glycoprotein on activated human iIELs and, potentially, other mucosal T lymphocytes is involved in the down-regulation of cytolytic function. The Journal of Immunology, 1999, 163: 1363–1370.

The biologic role of human intestinal intraepithelial lymphocytes (iIEL)³ and their functional relationship with the intestinal epithelial cell (IEC) remains incompletely characterized. Human iIELs have been shown to exhibit cytolytic and possibly immunoregulatory functions through the secretion of a variety of cytokines, suggesting an important role in local immunosurveillance of the IEC and the regional microenvironment (1). However, the molecules on the cell surface of iIELs and their IEC counter-receptors that regulate the functional activation of iIELs and may be used in this special microenvironment are only beginning to be elucidated.

A significant fraction of human iIELs of both the small and large intestine are CD8 αβ⁺ and CD45RO⁺ T cells that express a limited array of αβ and, to a lesser extent, γδ TCRs (2–5). These phenotypic properties indicate that most iIELs are memory cells that localize to the basolateral surface of IECs for the recognition of a limited number of Ags in the context of MHC class I or class I-like molecules on the IEC. However, the majority of iIELs in mouse and human are CD28⁻, suggesting that other costimulatory molecules for TCR/CD3 complex-mediated activation may be important in providing necessary secondary signals for iIEL activation (6–11). Candidate costimulatory molecules for human iIELs include CD2 (10), CD101 (8), BY-55 (9), and the αβ2 integrin (11), which are expressed by the majority of iIELs.

It has also become increasingly evident that in addition to activating costimulatory molecules, T cells can express a variety of molecules that deliver an inhibitory signal such that either the initial activation of the T cell is prevented or the activated state is down-regulated. The former type includes the killer inhibitory receptors (KIR), which are expressed on a subset of T cells and bind specific types of MHC class I molecules on the target cells (12). The latter type includes CTLA-4 (CD152) which, when expressed after T cell activation, binds either CD80 (B7.1) or CD86 (B7.2) on APC (13, 14). KIRs characteristically contain Ig-like extracellular domains and one or more immune receptor tyrosine-based inhibitory motifs (ITIM) in their cytoplasmic tails with a consensus sequence of I/L/VxYxxL/V (15). In the case of CTLA-4, the cytoplasmic tail contains the sequence GxYxxM, which is highly similar to, but not identical with, the ITIM of KIRs. ITIM-containing receptors function in the recruitment of either the Src homology-domain-containing protein tyrosine phosphatases, SHP-1 and SHP-2, or the SH2 domain-containing inositol polyphosphate 5-phosphatase, SHIP (16). These phosphatases function in the dephosphorylation of signaling molecules recruited by immune receptor tyrosine-based activation motif (ITAM)-bearing receptors such as those contained in the CD3-γ, -δ, -ε, and -ζ chains that associate with the TCR. As such, ITAM-bearing receptors on T cells are predicted to down-regulate activation events elicited by ITAM-bearing receptors if both are ligated in close proximity to one another. Importantly, neither KIRs and CTLA-4 nor CD80/1

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¹Gastroenterology Division, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115; ²Medical Research Council, Molecular Haematology Unit, John Radcliffe Hospital, Oxford, United Kingdom; ³Institut Fuer Medizinische Immunologie, Charité-Universität Berlin, Berlin, Germany; ⁴Imperial Cancer Research Fund-Medical Oncology Unit, St. Bartholomew’s Hospital Medical College, London, United Kingdom; ⁵Immunopathology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; and ⁶Combined Program in Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital and Harvard Medical School, and **Department of Adult Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

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CD86 have been observed on human iIELs or IECs of the intestine, respectively.

In this report we provide evidence in support of a novel role for biliary glycoprotein (BGP; CD66a), a member of the carcinoembryonic Ag family (CEA), as an inhibitory receptor for activated T cells contained within the human intestinal epithelium. These studies also suggest that, in a regional microenvironment that is predominantly CD28/CTLA-4-CD80/CD86 negative, other receptor-ligand interactions may provide the necessary down-regulatory signals to limit T cell activation and immunopathology.

Materials and Methods

Antibodies

The 34B1, 26H7, and 5F4 mAbs were produced by immunizing BALB/c mice with the activated human iIEL line, 191E, obtained from a subject with celiac disease as previously described (8). Hybridoma supernatants were screened by indirect immunoperoxidase staining of frozen intestinal and tonsillar tissue sections to identify and characterize the mAbs used in this report as previously described (17). The isotypes of 34B1 (IgG1), 26H7 (IgG1), and 5F4 (IgG1) were determined by ELISA using murine isotype-specific mAb (Boehringer Mannheim, Indianapolis, IN). OKT3 (IgG2a) is a mouse anti-human CD3 mAb (provided by Dr. Robert Fink-berg, Dana-Farber Cancer Institute, Boston, MA). TS 2/18 (provided by Dr. Lloyd Krickbühl, Brigham and Women’s Hospital) is an anti-CD2 mAb (mouse IgG2b). OKT4 (mouse IgG2b) and OKT8 (mouse IgG2a) are mAbs specific for human CD4 and CD8α, respectively (obtained from American Type Culture Collection, Manassas, VA). MA22 (CD66abce; clone 103A) was obtained from MA27 (CD66e; clone 26/51; IgG2a), MA28 (CD66e; clone 26/3/13; IgG1), MA30 (CD66c; clone 9A6; IgG1), MA41 (CD66b; clone BIRMA17c; IgG1), MA61 (CD66b; clone 80H3; IgG1), MA76 (CD66c; clone 12-140-4; IgG1), MA79 (CD66b; clone B13.9; IgG1), MA81 (CD66b; clone G10F5S; IgG1), MA83 (CD66c; clone b7.8.5; IgG1), MA84 (CD66d; clone COL-1; IgG2a), MA86 (CD66ace; clone B6.2; IgG1), and MA91 (CD66e; clone YG-C94G7; IgG1). These mAbs that were obtained from Cappel (West Chester, PA). mAbs were affinity purified with protein A or Sepharose G 200.

Cells and cell lines

Human iIELs were obtained, and cell lines EEI-10 (small intestine), EEI-5 (small intestine), KJ-3 (small intestine), and CLJ (large intestine) were generated from donors as previously described (18) and maintained by stimulation with 1 μg/ml PHA-P (Murex, Dartford, UK.) in RPMI 1640 containing 10% human serum (type AB; Sigma, St. Louis, MO), 5 U/ml PHA-P (Murex, Dartford, U.K.) in RPMI 1640, 1 μg/ml bacterial lipopolysaccharide (from Escherichia coli), and 2 mM L-glutamine (Life Technologies, Gaithersburg, MD) for 24 h at room temperature. For detection, biliary isolated mouse anti-mouse Ig (Vector Laboratories) was used, followed by incubation with alkaline phosphatase-labeled avidin reagent (Vector Laboratories) for 30 min. After development using the alkaline phosphatase substrate kit III (Vector Laboratories) for 15 min, the specimens were postfixed with 2% paraformaldehyde and mounted with Glycergel (Dako). Each step was followed by three washes with PBS. Incubation with 0.3% H2O2 in PBS was used to block endogenous peroxidase activity, and sequential incubations with avidin and biotin (Vector Laboratories) were used to block endogenous biotin.

Transfectants

The BGPx molecule was constructed as follows. The N-terminal domain and the transmembrane/cyttoplasmic domains of human BGPx were each amplified separately by PCR with the primer pairs BGPAMP-S (CATTCATCATCACTATGTATGTCGCCACTC) and NTM-AS (GCCGTTGGTCGGGAGCTCCGCGGTATAC) and NTM-S/(GTATACCCGGAGCTCGCCCAAAGAAAATGC/G3/BGP TRANS-CYT-AS) (ClonetACTACTACTCTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTAC. The isotypes of 34B1 (IgG1), 5F3 (IgG1), 5F4 (IgG1), and TS 2/18 were sequenced by standard methods. The BGPx cDNA was extracted and sequenced by standard methods. The BGPx cDNA was digested with EcoRI and NotI restriction enzymes and subcloned into the pcDNA1/Amp vector (Invitrogen, San Diego, CA). The BGPx cDNA in this vector and the pSV2neo plasmid (Clontech, Palo Alto, CA) were linearized with XhoI and BamHI, respectively, and electroporated into CHO cells at a ratio of 1:15, which was selected in G418 and on the FACS cell sorter to create a stable CHO-BGPx cell line as described previously (20). CHO cells stably transfected with BGPx, neomycin, BGC5 (21), and BGPx (22) and HeLa cells stably transfected with CEA, CGM1, NCA, and CGM6 have been previously described (20–22).

Flow cytometry

Flow cytometry was performed as previously described (2, 3). Staining was performed with 1 μg of the primary Ab followed by incubation with 1 μg of a goat anti-mouse FITC secondary Ab (Zymed, San Francisco, CA) with analysis on a FACScalibur (Becton Dickinson, Sunnyvale, CA) flow cytometer.

COS cell expression cloning

A cDNA library was constructed in the pCDM8 vector using poly(A)+ RNA from resting and activated human peripheral blood T cells (PBt) in the vector pAEXF (23). For the first round of selection, COS cells were transfected via the DEAE-dextran procedure (24) with 0.2 μg of library DNA/100-mm dish. After 48 h, cells were harvested, incubated with the anti-CD3 mAb (1/500 dilution of ascites), washed, and panned with anti-IgG1-coated plates as previously described (23–25). Episomal DNA was purified from adherent cells, reintroduced into CHO cells, and electroporated into CHO cells at a ratio of 1:15, which was selected in G418 and on the FACS cell sorter to create a stable CHO-BGPx cell line as described previously (20). CHO cells stably transfected with BGPx, neomycin, BGC5 (21), and BGPx (22) and HeLa cells stably transfected with CEA, CGM1, NCA, and CGM6 have been previously described (20–22).

Radiolabeling, immunoprecipitation, and electrophoresis

COS cells, 9 h after transient transfection, were removed nonenzymatically from plastic petri dishes, and iIELs were labeled with Na+[35S] by the lactoperoxidase-catalyzed method as previously described (26). Immunoprecipitations, digestion with N-glycanase, and SDS-PAGE were performed as previously described (26).
Production of soluble recombinant proteins and analysis of Ab binding

Details of the pIG vector (R&D Systems Europe, Abingdon, U.K.) containing the Fc genomic fragment of human IgG1 and incorporating the hinge (H), CH2, and CH3 domains and of the construction and purification of the CD66a-Fc soluble proteins containing the N, NA1B1, and NA1B1A2 extracellular domains, Muc-18-Fc (R&D Systems) and NCAM-Fc, have been described previously (20, 27, 28). Ab binding was quantified by ELISA with detection by alkaline phosphatase-conjugated goat anti-mouse Ig (Boehringer Mannheim, Indianapolis, IN) and paranitrophenyl phosphate (Sigma) as substrate as previously described (29).

Cytotoxicity assays

Cytotoxicity was evaluated as previously described (30). Briefly, the P815 mouse mastocytoma cell line was labeled with 100 μCi of 51Cr (New England Nuclear, Boston, MA) at 37°C for 30 min. The radiolabeled cells (2 × 105), in 100 μl of complete medium, were added to 100 μl of varying concentrations of effector T cells in 100 μl of complete medium in triplicate in a 96-well V-bottom plate. Before addition of target cells, the effector cells were incubated for 20 min at room temperature with medium, the OKT3 mAb (100 ng/ml of purified Ab), and/or varying concentrations of the 34B1 mAb, the 26H7 mAb, the 5F4 mAb as purified mAbs, or purified IgG1 Ab as a control (Sigma). Lymphokine-activated killer activity was assessed by examining cytotoxicity in the absence of OKT3 mAb. After 5 h, 100 μl of supernatant was removed for analysis in a gamma counter (LKB Wallac Clini Gamma 1272, Turku, Finland). Spontaneous and maximal release were measured by incubating target cells with medium or 1% Nonidet P-40, respectively. The percent cytotoxicity was calculated using the formula [experimental release − spontaneous release] × 100/[maximal release − spontaneous release].

Results

Identification of an Ag on IECs that is expressed by activated, but not resting, iIELs

During the development of iIEL-specific mAbs, obtained by immunizing mice with an iIEL T cell line from human small intestine propagated in vitro, it was observed that several of the mAbs stained IECs, as shown by immunohistology of normal human small and large intestines. Staining of human intestinal tissue sections showed that these three mAbs (34B1, 26H7, and 5F4) only stained IECs, not iIELs (Fig. 1, A–C). The in vivo tissue staining with these Abs appeared to be on the cell surface, as confirmed by flow cytometric analysis of a normal human IEC line, HT29 (data not shown). Because these three Abs did not stain iIELs in situ, as determined by immunohistology (Fig. 1, A–C), or immediately after isolation as determined by flow cytometry (Fig. 1D), it was predicted that iIELs, activated during the process of in vitro cultivation, expressed neoantigens that were constitutively expressed by IECs. Indeed, after maintenance in vitro as continuous cell lines with PHA-P activation in the presence of allogeneic feeder cells, the majority of iIELs expressed the Ag recognized by these three mAbs. Staining of an iIEL T cell line, EEI-5, established from the small intestine, that was 90% CD8+ and 10% CD4+ indicates that all iIELs expressed the Ag recognized by the three mAbs after this type of in vivo activation (Fig. 1E). Similar observations were made with an iIEL T cell line prepared from the large intestine, CLI, which was 40% CD8+, 30% CD4+, and 30% double negative (CD4−CD8−) at the time of staining available with the in vivo phenotype of iIELs in this tissue site (31) (data not shown). The expression of this Ag was observed within 7 days of in vitro activation of freshly isolated normal human iIELs, indicating that the observations were not an artifact of in vivo cultivation (Fig. 1F).

To determine whether this Ag was an activation Ag in vivo, two-color immunoperoxidase staining was performed on a case of active celiac disease. As shown in Fig. 2, numerous CD3+ 5F4− cells, which stained blue, consistent with T cells, were observed throughout the lamina propria and epithelia (open arrow). CD3− 5F4+ cells, which stained brown, included cells with the morphology of granulocytes (small arrow) and intestinal epithelial cells (large arrow). Rare double-positive cells (arrowheads) that were both blue and brown, consistent with 5F4-staining, CD3+ cells,

FIGURE 1. Identification of three mAbs (34B1, 5F4, and 26H7) that recognize IECs but not resting iIELs. A–C, Immunohistology of normal human large intestine stained with the 34B1 (A), 5F4 (B), and 26H7 (C) mAbs with binding detected by subsequent incubation with a goat anti-mouse HRP-conjugated Ab as described in Materials and Methods. The precipitated brown reaction product indicates specific staining on the enterocyte. Magnification, ×20. Staining with normal mouse serum was negative (data not shown). D, One-color flow cytometric analysis of iIELs freshly isolated from normal human small intestine with the 34B1 mAb, the OKT3 Ab (anti-CD3), or normal mouse serum (NMS) as a control. E, One-color flow cytometric analysis of an activated iIEL cell line derived from the small intestine, EEI-5, generated as described in Materials and Methods, with 34B1 mAb, 26H7 mAb, 5F4 mAb, or normal mouse serum (NMS) as a negative control. F, One-color flow cytometric analysis of freshly isolated iIELs from the small intestine, which are >90% CD8+, with NMS; a pool of the 34B1, 26H7, and 5F4 mAbs; the OKT3 Ab (anti-CD3); or the OKT8 Ab (anti-CD8) 7 days after in vitro stimulation with irradiated allogeneic feeder cells in the presence of PHA-P and rIL-2.
were observed in both the lamina propria and epithelium. These results suggest that the expression of this activation Ag is likely to be relevant to in vivo situations, because low numbers of mucosal T cells could be stained with the SF4 mAb in active celiac disease (Fig. 2). These phenotypic studies with iIELs from multiple donors and tissue sites (small intestine and colon) indicate that induction of this activation Ag is likely to be a common feature of human iIELs.

Identification of BGP as an activation Ag on human iIELs

To define the nature of this activation Ag, the 34B1 mAb was used to clone the cDNA that coded for the cognate Ag of the 34B1 mAb by COS cell expression cloning. Because the three mAbs (34B1, 26H7, and SF4) were also noted to stain activated T cells from human peripheral blood (data not shown), COS cells were transfected with a mixture of three cDNA libraries from resting and activated human PBTs. Transiently transfected COS cells were subjected to three rounds of panning with the 34B1 mAb. After the third round of panning, 17 of 50 randomly selected E. coli transformants contained plasmids with a 3.3-kb insert. The inserts in these plasmids were similar by restriction digest analysis. COS cells transfected with these plasmids were stained specifically with the 34B1 mAb.

One of these clones, pPAN3.1, was selected for further characterization. This plasmid directed the translation, when transfected into COS cells, of a 120-kDa glycoprotein that was specifically recognized by the 34B1 and SF4 mAbs and that resolved as major bands of 70 kDa and several minor bands of lower molecular mass after digestion with N-glycanase (Fig. 3). A similar glycoprotein was immunoprecipitated from radiolabeled cell surface iIEL proteins by all three mAbs (Fig. 3). Complete DNA sequencing of both strands of this cDNA revealed a sequence that was 97% identical with the b splice variant of BGP or CD66a (GenBank accession no. X14831), with all the differences occurring outside the coding region. Because the cDNA predicted a polypeptide backbone of 58 kDa, the data in Fig. 3 suggest that several of the carbohydrate modifications were relatively resistant to N-glycanase digestion.

BGP are members of the Ig supergene family, which consists of an N-terminal Ig V (IgV)-related domain, that is highly homologous to the N domains of other CEA or CD66 family members, followed by several IgC2-related domains, A1 and B1, and the A2, Y, or Z domains, which are unique to BGP isoforms (20, 21, 29, 32). To confirm that the 34B1-related mAbs were reactive with BGP and to define the specific protein domain to which these mAbs were directed, the Abs were tested in a binding assay with Fc fusion proteins containing the N domain of CD66a, NA1B1 domains of CD66a, the NA1B1A2 domains of CD66a, or N-CAM (CD56) as a negative control (Fig. 4A). As shown in Fig. 4B, these studies confirmed the recognition of BGP (CD66a) by the three mAbs and showed that all three mAbs reacted with the N domain.

To further confirm that the cognate Ag of the 34B1-related mAbs was BGP, the three mAbs were tested for their ability to stain CHO cells stably transfected with several splice variants of BGP (BGPa, BGPc, and BGPb) and HeLa cells transfected with other members of the CD66 serologic cluster, including CD66b (CEA gene-related member 1, CMG1), CD66c (CEA gene-related member 6, CGM6), CD66d (nonspecific cross-reacting Ag, NCA), and CD66e (CEA; Fig. 5). Except for CMG1 (CD66b), the 34B1 mAb stained all the CD66 family members tested, including all the...
FIGURE 5. Specificity of the anti-BGP mAbs for other CD66 family members. Flow cytometric analysis of BGPα, BGPγ, and BGPγ′ transfectants of CHO, and KEA, NCA, CCGM6, and CGM1 transfectants of HeLa cells compared with the mock (Neo) transfectants after staining with 34B1, 5F4, and 26H7 mAbs or isotype-matched IgG1 Ab as a negative control. All transfectants were positively stained with control mAbs specific for the transfected cDNA (data not shown).

CD66a splice variants. The 26H7 and 5F4 mAbs, however, were quite interesting in that they only stained the CD66a splice variants, suggesting that they were probably specific for the N domain of this molecule. Notably, there is only one other previous report of a CD66a-specific single-chain Ab fragment and no previously described CD66a-specific mAbs (33). These results clearly identify the N domain of BGP as the cognate Ag of the 34B1, 26H7, and 5F4 mAbs and show that the 34B1 mAb recognizes other CD66 forms. In addition, the 34B1, 26H7, and 5F4 mAbs are probably specific for a determinant contained within the polypeptide chain and not a carbohydrate side-chain modification, because these mAbs recognized the protein from tunicamycin-treated cell lines and specific mutations in the N domain-abrogated Ab binding to N domain mutants (S. Watt and R. Blumberg, unpublished observations).

Ligation of BGP inhibits the cytolytic function of iIELs

Our observation that BGP was expressed on activated iIELs, as defined by staining with the BGP-specific mAbs, 34B1, 26H7, and 5F4, was novel, because BGP has previously been primarily viewed as a molecule expressed on epithelial cells and granulocytes and involved in cell-cell adhesion and regulation of epithelial cell growth. In addition, BGP is the only CD66 isoform expressed by activated human iIELs. Fig. 6 shows the staining of an activated human iIEL cell line from the small intestine, EEI-10, with a panel of mAbs specific for CD66a–e. As shown, mAbs MA76 (CD66ae), MA86 (CD66ace), 34B1 (CD66ace), and 5F4 (CD66a), which are capable of recognizing CD66a-specific mAbs, but not mAbs specific for CD66b (MA41), CD66c (MA30), CD66e (MA27), or CD66de (MA84), exhibited significant staining. Similarly, the mAbs MA28 (CD66e), MA61 (CD66b), MA79 (CD66b), MA81 (CD66b), MA83 (CD66e), and MA91 (CD66e) did not stain the activated human iIEL cell line, EEI-10 (data not shown).

The function of BGP on iIELs and T cells, in general, is unknown. Importantly, the cytoplasmic tail of the BGPα and BGPβ splice variants, but not CD66b-e, contain two ITIM domains separated by 21 aa, raising the possibility that BGP might function as an inhibitory molecule on T cells (34, 35). A major function of activated iIELs is as cytolytic effector cells (1, 2). Therefore, the effects of the three mAbs on the cytolytic function of iIELs after 8–10 days of stimulation were examined in a redirected lysis assay in the presence of the OKT3 mAb. Fig. 7 shows a dose-finding study with the three CD66-specific mAbs described here at a range of concentrations. As predicted, all three Abs specifically inhibited the anti-CD3-directed cytolytic function of the iIEL cell lines. Inhibition of cytolytic activity required 100 μg/ml for the two highly specific mAbs (5F4 and 26H7), but only 10 μg/ml or less for the broadly reactive mAb (34B1). To confirm these results, the two highly specific Abs were examined at a concentration of 100.0 μg/ml at varying E:T cell ratios in comparison with an isotype-matched Ab with the KJ-3-3 cell line. As shown in Fig. 8A, neither the 5F4 nor the 26H7 mAbs directly stimulated the cytolytic activity of the iIELs. In addition, both the 5F4 and 26H7 mAbs, but not the control Ab, significantly inhibited the anti-CD3-directed lysis. The 5F4 mAb inhibited the lysis by 22, 35, and 38% at E:T cell ratios of 100:1, 50:1, and 25:1, respectively. Similarly, the 26H7 mAb inhibited the lysis by 21, 39, and 46% at E:T cell ratios of 100:1, 50:1, and 25:1, respectively. On the other hand, the inhibition by the control Ab at similar E:T cell ratios was 4, 5, and 5%. Moreover, the anti-CD3-directed cytolytic activity was not inhibited by a CD2-specific mAb, TS 2/18, which would be expected to inhibit CD58-like interactions with the P815 cell line, suggesting that the inhibition by the anti-CD66a mAbs was probably not due simply to an effect on adhesion (Fig. 8B).

In some experiments, when iIEL cell lines were harvested very soon (<6–7 days) after PHA-P stimulation in the presence of allogeneic feeders and cytokines, high levels of P815-directed cytolysis were observed in the absence of the anti-CD3 mAb consistent with lymphokine-activated killer activity, a response previously described for iIELs (36). As shown in Fig. 8C, this type of cytolytic activity was extremely sensitive to the inhibitory effects of all three Abs (34B1, 26H7, and 5F4) in comparison with an IgG1 control Ab, with up to 50% inhibition at a dose of 2 μg/ml of mAb. When cytolytic activity was assessed with a pool of the three Abs (2 μg/ml each), the lymphokine-activated killer activity-related cytolytic activity was strongly inhibited.
FIGURE 7. Abs specific for N domain of BGP inhibit anti-CD3-directed lytic activity of alloactivated human iIELs. EEI-10 and KJ-3 iIEL lines derived from normal human small intestine were tested in a redirected lysis assay using the P815 cell line as target in the presence of normal isotype-matched IgG1 (at 1, 5, 10, 20, or 100 \( \mu \text{g/ml} \)) with or without the anti-CD3 mAb, OKT3 (100 ng/ml). Medium indicates the cytotoxicity in the absence of added Abs. The figure shows the percent cytotoxicity at an E:T cell ratio of 50:1 for the EEI-10 cell line (top and middle panels) and the KJ-3 cell line (bottom panel). The vertical bars display the mean and SEM for all measurements. The concentration of purified Abs used for each coincubated Ab is shown for each condition. Significant differences between anti-CD3-directed lysis in the presence of anti-BGP mAb and either anti-CD3-directed lysis alone (OKT3) or anti-CD3-directed lysis in the presence of irrelevant IgG1 are indicated (*, IgG1 vs 34B1 at 1 \( \mu \text{g/ml} \), \( p = 0.0018 \); **, IgG1 vs 34B1 at 5.0 \( \mu \text{g/ml} \), \( p = 0.0252 \); †, IgG1 vs 34B1 at 10.0 \( \mu \text{g/ml} \), \( p = 0.0015 \); ‡, IgG1 vs 5F4 at 100.0 \( \mu \text{g/ml} \), \( p = 0.0002 \); §, OKT3 vs 26H7 at 100.0 \( \mu \text{g/ml} \), \( p = 0.0020 \)).

FIGURE 8. Characterization of inhibition of anti-CD3 directed and lymphokine-activated killer activity of iIELs by anti-BGP Abs. A, The anti-CD3-directed lysis of the KJ-3 iIEL cell line was examined as described in Materials and Methods in the absence (OKT3) or the presence of either the CD66a-specific mAbs, 5F4 or 26H7, or an isotype-matched IgG1 Ab at a concentration of 100.0 \( \mu \text{g/ml} \) and E:T cell ratios of 100:1, 50:1, and 25:1. Cytolysis in the absence of added Abs (medium) and that in the presence of the test Abs alone (26H7, 5F4, and control IgG1) are also shown. The SEM for each measurement is indicated. This figure is representative of six experiments. B, The anti-CD3-directed lysis of the P815 cell line was examined in either the presence or the absence of an isotype-matched irrelevant Ab (IgG), the TS2/18 mAb, or CD66a-specific mAbs, 26H7 and 5F4, at a concentration of 100 \( \mu \text{g/ml} \). P815 lysis in the absence of anti-CD3 with the panel of Abs is also shown. The inhibition by the CD66a-specific mAbs was statistically significant (26H7 + OKT3 vs TS2/18 + OKT3, \( p = 0.0095 \); 5F4 + OKT3 vs TS2/18 + OKT3, \( p = 0.0059 \)). C, The effects of an irrelevant IgG1 mAb (2 \( \mu \text{g/ml} \)); the anti-BGP-specific mAbs 34B1, 26H7, and 5F4 alone at a concentration of 2 \( \mu \text{g/ml} \) each; or a pool of the three mAbs (2 \( \mu \text{g/ml} \) each) on cytolyis of the P815 cell line by the EEI-10 iIEL cell line were examined 5 days after stimulation with PHA-P and irradiated allogeneic PBMCs. Cytotoxicity was assessed at an E:T cell ratio of 25:1. The medium control shows cytotoxicity in the absence of added Abs. The inhibition of cytotoxicity by the anti-BGP Abs was statistically significant (26H7, \( p = 0.048 \); 34B1, \( p = 0.004 \); pool, \( p = 0.0016 \)). This figure is representative of five experiments.
was inhibited by 70%. This finding suggested synergistic inhibition by the three mAbs and is consistent with recent findings that the three mAbs recognize distinct epitopes within the N domain, based upon an analysis of binding to N domain mutants (S. Watt and R. Blumberg, unpublished observations).

Discussion

Through the characterization of three mAbs raised against an activated iIEL cell line from the human small intestine, we have provided evidence for a potential role of human BGP (CD66a) as an inhibitory molecule on activated iIELs. These data are especially relevant to iIELs, as they suggest that other molecules, such as BGP, may contribute to down-regulation of T cell activation in the absence of CTLA-4. These studies are also of general interest given the observation that BGP is also expressed by human PBTs (37, 38).

Human BGP is a member of the CEA family of glycoproteins, part of the Ig supergene family, and encoded in a large cluster on chromosome 19 (20, 22, 28, 29, 32). The CEA cluster is highly related to the genetically linked, pregnancy-specific gene cluster (32, 35). The CEA subgroup of this family is serologically defined as CD66a (BGP or C-CAM), CD66b (CGM6), CD66c (NCA), CD66d (CGM1), and CD66e (CEA). These structurally related glycoproteins consist of a highly homologous membrane distal amino-terminal IgV-like N domain and variable numbers of membrane distal IgC2-like domains in the case of BGP, NCA, CGM6, and CEA. In contrast to human CEA, CGM6, and NCA, which are linked to the membrane by a glycosylphosphatidylinositol anchor, CGM7, CGM1, and BGP are type 1 transmembrane glycoproteins. The latter exist as isoforms containing short or long cytoplasmic tails.

BGP and its mouse and rat homologues C-CAM (35, 39, 40) have been regarded mainly as cell-cell adhesion and signaling molecules that are expressed primarily by epithelial cells of the gastrointestinal tract and biliary tree, neutrophils, and, more recently, B cells and human PBTs (37, 38, 41). Consistent with this we have observed that the mAbs described here stain epithelial cells in a tissue sample under normal conditions. More importantly, the function of BGP on epithelial cells may deliver a negative growth signal, which may be decreased during tumor formation due to diminished expression of BGP (44). BGP also exhibits a high degree of alternate transcriptional processing, resulting in at least eight potential alternate transcripts. Two of these transcripts, BGPα and BGPβ, encode a long cytoplasmic tail of 73 aa containing two ITIM motifs, which suggests a role as inhibitory receptors (35). Indeed, this cytoplasmic tail, when tyrosine phosphorylated, is capable of binding SHP-1 in a mouse colon carcinoma cell line (34). Such interactions may account for the inhibitory growth effect of this molecule on epithelial cells.

In this regard, taking advantage of several newly generated mAbs with unique specificity for BGP, we have been able to determine that BGP regulates CD3-directed and lymphokine-activated killer activity of activated human iIELs. In preliminary studies the Abs described here also inhibit the activation of human PBTs, suggesting that the results contained in this report may be extensible to T cells in general (data not shown). The mechanism of the inhibition of cytolyis is unknown. However, given the observations that the cytoplasmic tail of the BGPα and BGPβ splice variants contain the ITIM motif (34) and that the BGP homologue in mouse binds SHP-1 (33), it is possible that BGP on activated human iIELs interacts with intracellular phosphatases that down-regulate the function of ITAM-containing receptors such as CD3. Alternatively, BGP may function as an adhesion molecule that stabilizes effector cell interactions with the target such that blockade leads to diminished cytolyis. These hypotheses will be examined in future studies. Interestingly, the BGP gene maps to human chromosome 19q13.3 adjacent to the KIR locus on chromosome 19q13.4 (32, 45).

Although the ligand for BGP on the IEC is unknown, a good candidate is BGP itself or another CD66 family member in view of the known homophilic and heterophilic interactions among the CD66 group members (20, 21, 29, 35). These studies also suggest that BGP might provide inhibitory signals to iIELs in the absence of conventional inhibitory receptors such as KIRs and CTLA-4, which are notably absent from human CD8+ iIELs. It is also possible that the epithelial cell may actively regulate CD66a function and the capacity of the cell to mediate cytotoxicity, which would be highly relevant to epithelial cell infections, epithelial cell cancers, and chronic inflammatory diseases of the intestine. Indeed, we have observed CD66a expression on low numbers of mucosal T cells in celiac disease, as reported here.

The possibility that BGP might inhibit cytolytic T cell function during epithelial cell-T cell interactions extends the function of BGP to immunoregulation, making this the second example of a CD66 family member potentially involved in epithelial cell-T cell interactions. Mayer and colleagues have recently provided strong evidence for a role of a novel CD66e-related molecule, gp180, in directly ligating CD8 and activating p56Lck on T cells (46). In conclusion, our studies strongly suggest a much larger role for CD66 family members in regulating T cell activation and deactivation.

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