INFECTION AND IMMUNITY, Jan. 2009, p. 549–556 Vol. 77, No. 1
0019-9567/09/$08.00+0 doi:10.1128/IAI.01002-08

Characterization of *Entamoeba histolytica* Intermediate Subunit Lectin-Specific Human Monoclonal Antibodies Generated in Transgenic Mice Expressing Human Immunoglobulin Loci[▽]

Hiroshi Tachibana,[1]* Xun-Jia Cheng,[1] Hideo Tsukamoto,[2] and Johbu Itoh[2]

Department of Infectious Diseases[1] and Teaching and Research Support Center,[2] Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan

Received 11 August 2008/Returned for modification 23 September 2008/Accepted 31 October 2008

Four fully human monoclonal antibodies (MAbs) to *Entamoeba histolytica* intermediate subunit lectin (Igl) were prepared in XenoMouse mice, which are transgenic mice expressing human immunoglobulin loci. Examination of the reactivities of these MAbs to recombinant Igl1 and Igl2 of *E. histolytica* showed that XEhI-20 {immunoglobulin G2(κ) [IgG2(κ)]} and XEhI-28 [IgG2(κ)] were specific to Igl1, XEhI-B5 [IgG2(κ)] was specific to Igl2, and XEhI-H2 [IgM(κ)] was reactive with both Igls. Gene analyses revealed that the V_H and V_L germ lines were VH3-48 and L2 for XEhI-20, VH3-21 and L2 for XEhI-28, VH3-33 and B3 for XEhI-B5, and VH4-4 and A19 for XEhI-H2, respectively. Flow cytometry analyses showed that the epitopes recognized by all of these MAbs were located on the surfaces of living trophozoites. Confocal microscopy demonstrated that most Igl1 and Igl2 proteins were colocalized on the surface and in the cytoplasm, but different localization patterns in intracellular vacuoles were also present. The preincubation of trophozoites with XEhI-20, XEhI-B5, and XEhI-H2 caused significant inhibition of the adherence of trophozoites to Chinese hamster ovary cells, whereas preincubation with XEhI-28 did not do so. XEhI-20, XEhI-B5, and XEhI-H2 were injected intraperitoneally into hamsters 24 h prior to intrahepatic challenge with *E. histolytica* trophozoites. One week later, the mean abscess size in groups injected with one of the three MAbs was significantly smaller than that in controls injected with polyclonal IgG or IgM isolated from healthy humans. These results demonstrate that human MAbs to Igls may be applicable for immunoprophylaxis of amebiasis.

Amebiasis caused by infection with *Entamoeba histolytica* is one of the most problematic parasitic diseases of humans worldwide. It is estimated to result in 50 million cases of colitis and liver abscess and up to 100,000 deaths annually (54). However, an effective vaccine or chemoprophylaxis to prevent amebiasis has not been developed. The adherence of *E. histolytica* trophozoites to colonic mucins and various host cells is an essential event for colonization, invasion, and subsequent pathogenesis. The adherence is mediated by a galactose (Gal)- and *N*-acetyl-D-galactosamine (GalNAc)-inhibitable lectin (39). The lectin is a 260-kDa heterodimeric glycoprotein composed of a 170-kDa heavy subunit (Hgl) and a 31- or 35-kDa light subunit (Lgl) (38), and the Hgl is a candidate vaccine for amebiasis (22, 27, 30). We have demonstrated previously that a 150-kDa intermediate subunit (Igl), which is noncovalently associated with Hgl, also contributes to adherence (13). A mouse monoclonal antibody (MAb) specific for Igl significantly inhibits the adherence and cytotoxicity of trophozoites to mammalian cells and inhibits erythrophagocytosis (10, 48, 51). The immunization of hamsters with native Igl can inhibit amebic liver formation (11). There are two isoforms of Igl, which have 1,101 and 1,105 amino acids and are referred to as Igl1 and Igl2, respectively (9). The Igls are known to be cysteine-rich proteins containing multiple CXXC motifs, but the association between inhibitory effects and each Igl isoform is not well understood.

Recent studies have shown that cellular immunity is important for the prevention of invasive amebiasis (26, 42). However, it has been reported previously that passive immunization with rabbit antiserum to a serine-rich *E. histolytica* protein, with human anti-*E. histolytica* antibodies obtained from patients with amebic liver abscesses, or with a mouse MAb to a surface lipophosphoglycan antigen inhibits amebic liver abscess formation in a severe combined immunodeficient mouse model (31, 43, 55). We have also demonstrated previously that mouse MAb to Igl can inhibit liver abscess formation in hamsters (12). Therefore, human MAbs to these antigens may be applicable to reduce mortality from amebiasis by passive immunization. Hybridoma technology has been relatively unsuccessful for the generation of human MAbs, but several new methods have recently been developed (3, 5, 53), including the use of XenoMouse mice, which are transgenic mice containing the megabase-sized human immunoglobulin loci (17, 18, 32). Several human MAbs generated using this approach are now in clinical trials (4, 14, 28, 37).

In the present study, we used XenoMouse mice to generate fully human MAbs to *E. histolytica* Igl. Here, we report the molecular characterization of human MAbs specific for Igl1 and Igl2 of *E. histolytica*, and we also evaluate the effects of these human MAbs on amebic adherence in vitro and amebic liver abscess formation in hamsters.

MATERIALS AND METHODS

Cultivation of parasites. Trophozoites of the *E. histolytica* HM-1:IMSS strain were axenically cultured in TYI-S-33 medium supplemented with 15% adult

* Corresponding author. Mailing address: Department of Infectious Diseases, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan. Phone: 81 (463) 93-1121. Fax: 81 (463) 95-5450. E-mail: htachiba@is.icc.u-tokai.ac.jp.

▽ Published ahead of print on 10 November 2008.

bovine serum at 37°C (15). Cultured trophozoites were harvested in the logarithmic phase of growth and used in subsequent experiments.

Production of MAbs. XenoMouse 2a3 strain male mice, which express human immunoglobulin M (IgM), IgG2, and immunoglobulin κ genes, were provided by Abgenix, Inc. (Fremont, CA). Native Igl was purified from trophozoites of *E. histolytica* HM-1:IMSS by immunoaffinity column chromatography using mouse MAb EH3015 (13). XenoMouse mice were immunized intraperitoneally with 10 μg of Igl emulsified in complete Freund's adjuvant. The mice then received two booster inoculations of Igl in incomplete Freund's adjuvant at 2-week intervals. After an additional 4 weeks, the mice received Igl only. On day 4 thereafter, spleen cells were isolated and fused with X63-Ag8.653 mouse myeloma cells in 50% polyethylene glycol 1500. Hybridomas secreting MAbs against *E. histolytica* trophozoites were screened by immunofluorescent staining and cloned by limiting dilution. Immunoglobulin isotypes of MAbs were determined by immunofluorescent staining using subtype-specific secondary antibodies. Hybridomas were finally cultured in GIBCO hybridoma serum-free medium (Invitrogen, Carlsbad, CA). IgG and IgM were purified from the culture supernatants using a HiTrap protein G FF column (GE Healthcare, Buckinghamshire, England) and HiTrap SP Sepharose FF and HiLoad Superdex 200 columns (GE Healthcare), respectively. IgG and IgM fractions from sera of healthy individuals were also purified and used as controls.

Cloning and sequencing of immunoglobulin genes. Total RNA was purified from hybridomas and subjected to reverse transcription-PCR as described previously (47). Genes coding for the light (κ) chain and the Fd regions of the heavy (γ and μ) chains were amplified by 30 cycles of PCR. The light-chain genes were first ligated with an expression vector, pFab-His2, and introduced into *Escherichia coli* JM109 cells. The vector with inserts was then ligated with the Fd heavy-chain genes and introduced into *E. coli* cells. The production of Fab fragments to *E. histolytica* was screened by immunofluorescent staining (47). The light-chain and Fd heavy-chain genes from positive clones were subcloned into sequencing vectors and then sequenced.

Immunofluorescent staining for screening. Indirect immunofluorescent staining of fixed *E. histolytica* trophozoites was performed as described previously (50), except that 4% paraformaldehyde was used as the fixative. Fluorescein isothiocyanate (FITC)-conjugated goat antibodies to human IgG(H+L), human IgG Fab, human IgG2, and human IgM (ICN Pharmaceuticals) were used as secondary antibodies.

Confocal microscopy. Trophozoites of the *E. histolytica* HM-1:IMSS strain were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min and attached to silane-coated glass slides by using a Cytospin 2 centrifuge (Shandon, Oakland, CA). After being washed with PBS, the glass slides were incubated with 10% sucrose in PBS for 1 h and then stored at −80°C until use. The trophozoites were treated with 0.05% Triton X-100 in PBS for 5 min. After being washed with PBS, the trophozoites were blocked with 3% bovine serum albumin in PBS for 30 min and then incubated for 1 h at room temperature with labeled MAbs. An Alexa Fluor 488 protein labeling kit and an Alexa Fluor 594 protein labeling kit (Molecular Probes, Eugene, OR) were used for the labeling of MAbs. After being washed, the stained trophozoites were mounted using glycerol containing 1.25 mg of 1,4-diazabicyclo(2,2,2)octane/ml and 10% PBS, and the samples were observed using a Zeiss LSM510 META confocal laser scanning microscope.

SDS-PAGE and Western immunoblot analysis. Trophozoites of *E. histolytica* HM-1:IMSS were solubilized with equal volumes of sample buffer (29) containing 2 mM phenylmethylsulfonyl fluoride, 2 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, and 4 μM leupeptin for 5 min at 95°C. After centrifugation, the supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Western immunoblot analysis was performed as described previously (49). Horseradish peroxidase (HRP)-conjugated goat antibody to human IgG(H+L) (ICN Pharmaceuticals) was used as the second antibody. A Konica immunostaining HRP-1000 kit was used for development.

Preparation of recombinant Igls. An *E. histolytica* Igl1 fragment lacking only the N- and C-terminal signal sequences (thus comprising amino acids [aa] 14 to 1088) and three additional fragments of *E. histolytica* Igl1 (the N-terminal region [aa 14 to 382], the middle region [aa 294 to 753], and the C-terminal region [aa 603 to 1088]) were prepared in *E. coli* as described previously (46). For the preparation of an *E. histolytica* Igl2 fragment encompassing the full-length protein except for the signal sequences, a DNA fragment was obtained by PCR amplification of genomic DNA (HM-1:IMSS strain) with primers 5′-CCC TCG AGG ATT ATA CTG CTG ATA AAC TCA TTA ATA ACC-3′ and 5′-CCC TCG AGT TAA GTT TTG CAT GGC CCA TC-3′. Three DNA fragments encoding the N-terminal region (aa 14 to 382), the middle region (aa 294 to 757), and the C-terminal region (aa 604 to 1092) of *E. histolytica* Igl2 were also obtained by PCR amplification of DNA encoding full-length Igl2. The primers used were as follows: 5′-CCC TCG AGG ATT ATA CTG CTG ATA AAC TCA TTA ATA ACC-3′ and 5′-CCC TCG AGT TAA AGT TTG CAT GGC CCA TC-3′ for the N terminus, 5′-CCC TCG AGA CAG AAG AAA ATA AAT GTA-3′ and 5′-CCC TCG AGT TAA GAA CTT TGG TCA GTG-3′ for the middle region, and 5′-CCC TCG AGG AAG GAC TAA ATG CAG AAG AT-3′ and 5′-CCC TCG AGT TAA ATG CCT TTA GCT CCA TT-3′ for the C terminus. These DNA fragments were digested with XhoI, purified, and ligated with pET19b vector (Novagen, Madison, WI). The expression, purification, and refolding of the recombinant *E. histolytica* Igl2 proteins were performed as described previously (46).

Dot immunoblot analysis. Recombinant Igls were blotted onto nitrocellulose membranes. Membrane strips were blocked with 3% bovine serum albumin in PBS and allowed to react with antibodies for 30 min. After being washed with PBS containing 0.05% Tween 20, the strips were incubated with HRP-labeled goat antibody to human IgG(H+L) for 30 min. The strips were washed with PBS containing 0.05% Tween 20 and developed with a Konica immunostaining HRP-1000 kit. Recombinant Igls were also heat treated for 5 min at 95°C with or without 2-mercaptoethanol. These proteins were blotted onto nitrocellulose membranes and analyzed as described above.

Measurement of affinity constants. The affinity constants of the antibodies were assessed by surface plasmon resonance using a model 3000 instrument and general procedures outlined by the manufacturer (Biacore AB, Uppsala, Sweden). Recombinant Igls were immobilized onto a CM5 chip (Biacore) at a low density. Association and dissociation constants were determined using BIAevaluation 3.1 software (Biacore).

Flow cytometry. Intact trophozoites of *E. histolytica* HM-1:IMSS were incubated on ice with 3% bovine serum albumin in PBS for 15 min and then with MAbs for 15 min. After being washed with ice-cold PBS, the cells were incubated with a FITC-conjugated goat antibody to human IgG(H+L) for 15 min on ice. The cells were washed with ice-cold PBS and then fixed in 4% paraformaldehyde. Aliquots of approximately 5,000 cells per sample were analyzed using a FACSCalibur system (Becton Dickinson), with data analysis by CellQuest software (BD Immunocytometry Systems).

Adherence assay. The adherence of *E. histolytica* to Chinese hamster ovary (CHO) cells was examined as described previously (10). Briefly, trophozoites (10^4 cells) of the HM-1:IMSS strain were incubated with 10 μg of each MAb for 1 h at 4°C, washed with cold PBS, and then suspended in Ham's F-12 nutrient mixture containing 1% adult bovine serum. The trophozoites were mixed with CHO cells (2×10^5) in 1 ml of Ham's F-12 nutrient mixture, and the mixture was centrifuged at $150 \times g$ for 2 min and then incubated for 2 h at 4°C. After the removal of 0.8 ml of supernatant, the remaining pellet was subjected to a gentle vortex for 5 s and the number of trophozoites with at least three adherent CHO cells was determined by examining 300 trophozoites. Statistical analysis was performed by Student's *t* test.

Passive immunization and hepatic challenge. Male Syrian hamsters weighing 95 to 110 g were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Each hamster received an intraperitoneal injection of 0.5 ml of PBS containing 5 mg of MAb 24 h before challenge. Control hamsters received 0.5 ml of PBS only or 0.5 ml of PBS containing 5 mg of human polyclonal IgG or IgM. All the hamsters were anesthetized by intraperitoneal injection with pentobarbital, and then 10^5 trophozoites of the *E. histolytica* SAW755CR strain were inoculated into the left lobe of the liver. The hamsters were sacrificed 7 days after inoculation to examine the formation of amebic liver abscesses. The percentage of abscessed liver was calculated as the weight of the abscess divided by the liver weight recorded before abscess removal. Statistical analysis was performed by Student's *t* test.

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this paper have been deposited in the DDBJ, EMBL, and GenBank databases under accession numbers AB453230 to AB453237.

RESULTS

Specificity of human MAbs. Four human MAbs designated XEhI-20, XEhI-28, XEhI-B5, and XEhI-H2 were used in the study. Indirect immunofluorescent antibody staining using subtype-specific secondary antibodies showed that three of the MAbs, XEhI-20, XEhI-28, and XEhI-B5, were IgG2, while XEhI-H2 was IgM. The reactivities of the human MAbs to recombinant Igl1 and Igl2 from *E. histolytica* were examined by dot blot analysis. XEhI-20 and XEhI-28 were specifically reactive with Igl1, whereas XEhI-B5 was specific to Igl2. The

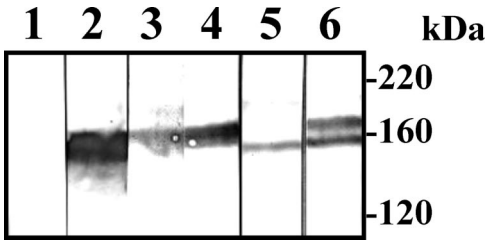

FIG. 1. Western immunoblot analysis of human MAbs to *E. histolytica*. Lysates of trophozoites from the HM-1:IMSS strain were subjected to SDS-PAGE in a 7.5% gel under nonreducing conditions and transferred onto polyvinylidene difluoride membranes. The strips were treated with the following: lane 1, sera from preimmune XenoMouse mice; lane 2, sera from XenoMouse mice immunized with native *E. histolytica* Igl; lane 3, XEhI-28; lane 4, XEhI-20; lane 5, XEhI-B5; and lane 6, XEhI-H2. HRP-conjugated goat antibody to human IgG(H+L) was used as a secondary antibody. The numbers on the right indicate molecular masses of size markers.

remaining MAb, XEhI-H2, was reactive with both Igls, as well as with sera from immune XenoMouse mice, indicating the presence of a common epitope in these proteins. The reactivities of the MAbs to native proteins were also examined by Western immunoblot analysis (Fig. 1). Under nonreducing conditions, immune sera and XEhI-H2 recognized two bands with apparent molecular masses of 150 and 170 kDa. XEhI-20 and XEhI-28 were reactive with the 170-kDa band, and XEhI-B5 was reactive with the 150-kDa band, indicating that the upper band was Igl1 and the lower band was Igl2.

Primary structures and gene usage patterns of human MAbs. Immunoglobulin genes from hybridomas secreting human MAbs were cloned and sequenced. As shown in Fig. 2, the primary structures of both the heavy and light chains of the four MAbs were different. However, similarity between the complementarity-determining regions in the light chains of XEhI-20 and XEhI-28 was found, while the complementarity-determining regions in the heavy chains of these two MAbs were quite different. Sequence analysis of the constant regions of the heavy chains also showed that XEhI-H2 was IgM. The sequence homology of these MAbs to germ lines was analyzed using the IgBLAST program. Three V segments of the heavy chains belonged to the family VH3, but their germ lines were different, and the remaining one (in XEhI-H2) was a member of the VH4 family (Table 1). The D segments differed among these MAbs, but the J segments were of the JH4 family in all except XEhI-20. The germ lines of both the V and J segments of the light chains in XEhI-20 and XEhI-28 were identical.

Affinity of human MAbs. The affinity of human MAbs to Igl was evaluated by surface plasmon resonance. The dissociation constants of the four MAbs ranged from 1.24×10^{-8} to 1.77×10^{-10} M (Table 2). The affinity of XEhI-28 was clearly lower than those of the other MAbs.

Epitopes recognized by human MAbs. Living trophozoites were incubated with MAbs, fixed, and then subjected to flow cytometry analysis. As shown in Fig. 3, all of the MAbs were reactive with the cells, suggesting that the epitopes exist on the cell surface. The reactivities of MAbs to partial fragments of *E. histolytica* Igl1 were also examined by dot blot analysis. XEhI-28 was reactive with the Igl N-terminal fragment (aa 14 to 382), but not with the middle region (aa 294 to 753) or the Igl C-terminal fragment (aa 603 to 1088). In contrast, XEhI-H2 was reactive with the Igl middle and C-terminal fragments, suggesting the localization of the epitope between aa 603 and 753. Since XEhI-20 and XEhI-B5 did not react with any partial fragments, these MAbs seem to recognize a particular conformation of Igl. To further evaluate the effect of the protein conformation on the binding of MAbs, recombinant

Heavy chain

```
                        FR1                 CDR1       FR2              CDR2
XEhI-20-VH   QVQLVESGGGLVQPGGSLRLSCAASGFTFS TYGMN WVRQAPGKGLEWIS FISSSSSTVYYADSVKG
XEhI-28-VH   E..........K..................  S.N..  ............VA S..H...YMFH...L..
XEhI-B5-VH   .....Q....V....R.......T.E....  SHA.H  ............VA V.WYDGVNT........
XEhI-H2-VH   ....QQ..P...K.SET.S.T.TV..GSIN N.YWS  .I..PA.......G R.-Y..GSTN.NP.L.S

                        FR3                          CDR3           FR4
XEhI-20-VH   RFTMSRDNAKNSLYLQMNSLRDEDTAVYYCAR AHYDWNFD-GM--DV WGQGTTVTVSS
XEhI-28-VH   ...I..........H.....A..M.......  DGSR-----.WYF.Y  .....L.....
XEhI-B5-VH   ...I....S..T.........A..........  EGLRFLEHIRGYF.Y  .....L.....
XEhI-H2-VH   .V...V.TS..QFS.KLS.VTAA.........  EVT----H----F.Y  .....L.....
```

Light chain

```
                     FR1                 CDR1             FR2          CDR2
XEhI-20-VL   EIVMTQSPATLSMSPGERATLSC RASQSISS-----NF-A WYQQKPGQSPRLLIY GASTRAT
XEhI-28-VL   ...L........V..........  .....V..-----.-L.  ........A......  .......
XEhI-B5-VL   D.EL....DS.AV.L.....IN. KS...VFYNSINK..L.  ....R...P.K.... W.....S
XEhI-H2-VL   D.......LS.PVT...P.SI.. .S...LLH-SNGF..LD  ..LR......Q.... LG.N..S

                        FR3                  CDR3        FR4
XEhI-20-VL   GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYNNWPLT FGGGTKVEIKR
XEhI-28-VL   ......N.G....D...S...P...N....H. .H.......  ...........
XEhI-B5-VL   .V.D.........D.........A..V.....  ...Y.T.P.  ..Q........
XEhI-H2-VL   .V.D.........D...K..RVEA..IG....  M.ALESLP.  ..Q........
```

FIG. 2. Deduced amino acid sequences corresponding to genes coding for heavy- and light-chain variable regions in human MAbs to *E. histolytica* Igls. FR, framework regions; CDR, complementarity-determining regions. Dashes and dots indicate deletions and identical residues, respectively.

TABLE 1. Comparison of gene usage patterns for heavy- and light-chain variable regions of anti-*E. histolytica* Igl human MAbs

MAb	Heavy chain						Light chain			
	V segment		D segment		J segment		V segment		J segment	
	Closest germ line	% Identity	Closest germ line	% Identity	Closest germ line	% Identity	Closest germ line	% Identity	Closest germ line	% Identity
XEhI-20	VH3-48	97	D3-10	100	JH6	87	L2	98	Jκ4	100
XEhI-28	VH3-21	95	D6-19	93	JH4	96	L2	94	Jκ4	100
XEhI-B5	VH3-33	97	D3-3	100	JH4	98	B3	96	Jκ1	100
XEhI-H2	VH4-4	97	D4-17	100	JH4	98	A19	94	Jκ1	100

Igls were heat treated under nonreducing and reducing conditions and then their reactivities to MAbs were compared with those of native recombinant Igls (Fig. 4). Reactivities to all MAbs were reduced by heat treatment, indicating that the conformation is important for all the epitopes recognized by these MAbs. Reactivity with XEhI-20 decreased clearly under reducing conditions, suggesting that an S-S bond is involved in the epitope. In contrast, the reactivity of XEhI-H2 with Igl was relatively retained after heat treatment under reducing conditions.

Localization of Igl1 and Igl2. *E. histolytica* trophozoites were double stained with XEhI-20 and XEhI-B5 and then observed by confocal microscopy (Fig. 5). Both Igl1 and Igl2 were detected on the plasma membrane and in the cytoplasm in all trophozoites. Igl1 and Igl2 were colocalized in most cells, but different localization patterns on vacuoles in the cytoplasm were also seen (Fig. 5D).

Inhibitory effects of human MAbs on amebic adherence. To examine the effect of human MAbs on amebic adherence, trophozoites were pretreated with 10 μg of human MAbs and then incubated with CHO cells. XEhI-20, XEhI-B5, and XEhI-H2 significantly inhibited amebic adherence compared with that of controls treated with PBS only (P, <0.0001 for each MAb), whereas XEhI-28 failed to produce inhibition (Fig. 6). The inhibitory effect of XEhI-B5 was significantly greater than those of XEhI-20 and XEhI-H2 (P, 0.0029 and 0.0284, respectively). The effect of XEhI-H2 was also significantly greater than that of XEhI-20 ($P = 0.0212$).

Effects of human MAbs on liver abscess formation. The three human MAbs showing neutralizing activity toward amebic adherence in vitro were further examined for their effects on amebic liver abscess formation in an animal model (Fig. 7). All of 12 hamsters treated with PBS only developed liver abscesses following infection with *E. histolytica*, with a mean percentage of abscessed liver of 42.7%. In two other control groups receiving 5 mg of IgG or IgM, the mean percentages of abscessed liver were 39.7 and 45.4%, respectively. When hamsters were passively immunized with 5 mg of XEhI-20, XEhI-B5, or XEhI-H2 24 h before infection, the mean percentage of abscessed liver was 13.9, 14.7, or 20.9%, respectively, and these values were significantly lower than those for animals immunized with isotype controls (XEhI-20 versus IgG control, $P < 0.0001$; XEhI-B5 versus IgG control, $P < 0.0001$; XEhI-H2

TABLE 2. Association (K_A) and dissociation (K_D) constants for the binding of human MAbs to recombinant Igls of *E. histolytica* as measured by surface plasmon resonance

MAb	Ligand	K_A (M^{-1})	K_D (M)
XEhI-20	Igl1	2.34×10^9	4.28×10^{-10}
XEhI-28	Igl1	8.03×10^7	1.24×10^{-8}
XEhI-B5	Igl2	5.65×10^9	1.77×10^{-10}
XEhI-H2	Igl2	2.26×10^9	4.43×10^{-10}

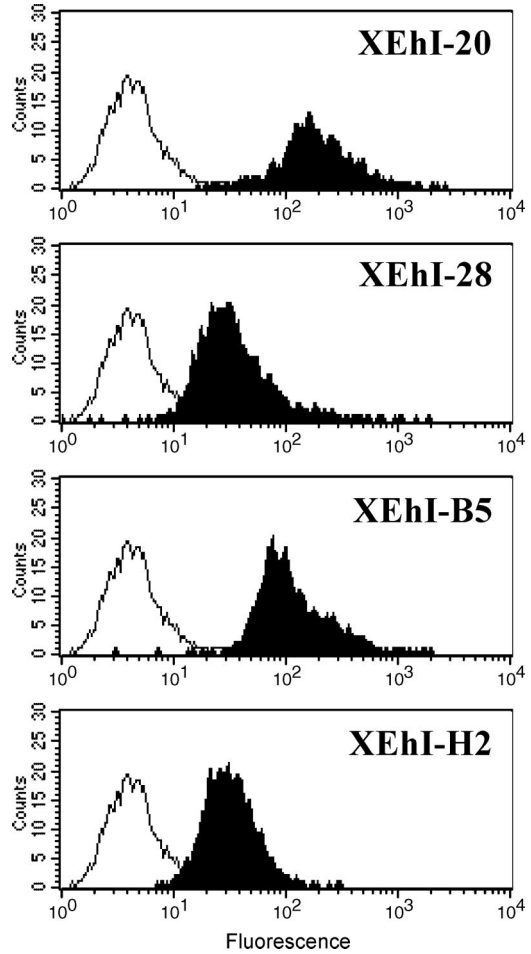

FIG. 3. Flow cytometric analysis of *E. histolytica* trophozoites stained with human MAbs to Igls (black-filled histograms). Intact trophozoites were incubated with human MAbs XEhI-20, XEhI-28, XEhI-B5, and XEhI-H2, followed by FITC-conjugated goat antibody to human IgG(H+L). The control was stained only with a secondary antibody (unfilled histograms). A representative histogram for each antibody is depicted. Fluorescence levels are expressed in arbitrary units.

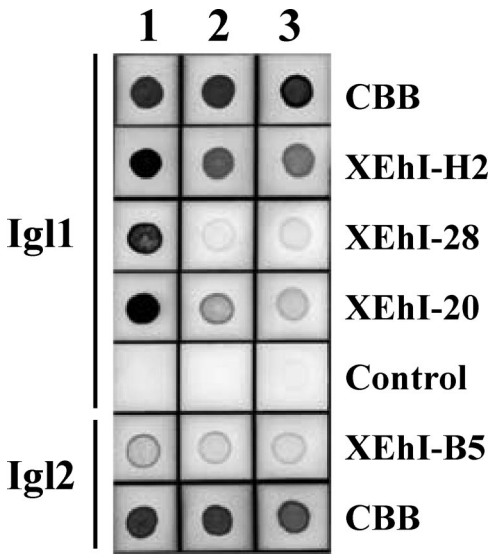

FIG. 4. Reactivity of human MAbs to recombinant Igls of *E. histolytica* in a dot blot analysis. Igl1 and Igl2 (500 ng each) were spotted onto nitrocellulose membranes (lane 1). The same amounts of Igls were spotted after heat treatment under nonreducing (lane 2) and reducing (lane 3) conditions. Two strips were stained with Coomassie brilliant blue (CBB), and other strips were treated with MAbs XEhI-H2, XEhI-28, XEhI-20, and XEhI-B5 or PBS (control). HRP-conjugated goat antibody to human IgG(H+L) was used as a secondary antibody.

versus IgM control, $P = 0.0003$). In the group treated with XEhI-20, no abscesses were found in three hamsters.

DISCUSSION

In the present study, human MAbs specific for Igl1 and Igl2 were successfully generated in XenoMouse mice. This is the first report concerning the production of fully human MAbs against *E. histolytica*. Native Igl purified using mouse MAb EH3015 was used for immunization, and the results indicated that the native Igl fraction contained both Igl1 and Igl2. In our previous study, SDS-PAGE analysis of the native Igl gave two bands with molecular masses of 150 and 170 kDa (11, 13). Western immunoblot analysis has also shown that MAb EH3015 recognizes 150- and 170-kDa molecules in the H-302:NIH strain of *E. histolytica* (48). The present study revealed that the 170-kDa band is Igl1 and the 150-kDa band is Igl2, although the molecular mass calculated from the DNA sequence is larger for Igl2 than for Igl1 (9).

Neutralizing activity toward amebic adherence to CHO cells was detected for both Igl1- and Igl2-specific human MAbs. Therefore, both Igls must be involved in amebic adherence. Since XEhI-28 did not affect amebic adherence to CHO cells, the N terminus of Igl1 may not be important for adherence to host cells. When the conformation of the recombinant Igls was changed by heat treatment, reductions in the reactivities of all MAbs were observed. Therefore, the epitopes recognized by these MAbs seem to be discontinuous. Indeed, XEhI-20 and XEhI-B5 reacted with full-length Igl molecules but failed to react with three partial fragments. Since the reactivity of XEhI-H2 was relatively retained after heat treatment under reducing conditions, we cannot exclude the possibility that its epitope is continuous. The presence of many cysteine residues in Igls may be important for the maintenance of their active conformations.

Confocal microscopy analysis demonstrated that the two Igls colocalized on the plasma membrane. However, it was also of interest that Igl1 and Igl2 showed different localization patterns in some intracellular vacuoles. This finding is in accord with the observation for *E. dispar* in a previous study using mouse MAbs specific for Igl1 and Igl2 of *E. dispar* (45). Recently, it has been reported that phagosomes of *E. histolytica* contain Igl and that the quantity of Igl varies during the maturation of the phagosome (35, 36). In addition, the expression level of the Igl1 gene is seven times higher than that of the Igl2 gene when the levels are compared by real-time PCR analysis (45). Although the primary structures of Igl1 and Igl2 are similar, these observations suggest that the two Igls may have different functions and different levels of expression during phagosome maturation, and the specific MAbs generated in this study may be useful for determining the associations of Igl1 and Igl2 with phagocytosis. We also note that different localization patterns of the two isotypes of the Rab7 small GTPase, *E. histolytica* Rab7A and *E. histolytica* Rab7B, in lysosomes and phagosomes have been reported previously (40).

We have generated several human MAb Fab fragments using combinatorial immunoglobulin gene libraries prepared from peripheral lymphocytes of a patient with an amebic liver abscess and asymptomatic cyst passers (8, 47, 52). Several Fabs recognize Hgl and are able to inhibit amebic adherence to host cells in vitro. However, we suspect that the in vivo effect of Fab fragments to *E. histolytica* trophozoites will be restricted because of the lack of an Fc region. Since the MAbs described in this paper are complete immunoglobulins, the effects of human MAbs on *E. histolytica* trophozoites in vivo may be induced with complement activation through the classical pathway (19). Indeed, it is well-known that IgM, in particular, is a mediator of complement activation. However, it has also been reported previously that Hgl of *E. histolytica* inhibits the assembly of C8 and C9 into the C5b-C9 membrane attack complex, thereby preventing complement-mediated lysis of the parasite (6). Recently, a cohort study in Bangladesh demonstrated that the mucosal IgA response directed to Hgl is linked to protection from both infection and disease (20, 21). In contrast, serum IgA has not been correlated with infection. Therefore, it is suggested that the systemic anti-Hgl antibody response may not offer direct protection from amebiasis (19). The details of the humoral immune response to Igl are unknown, but we have observed that serum antibodies to Igl are detected not only in symptomatic patients but also in asymptomatic *E. histolytica* cyst passers (46). In addition, sera from hamsters immunized with native Igl can inhibit in vitro amebic adherence to CHO cells by 98% at a 1:10 dilution (11).

In our previous study, the incidence of amebic liver abscess in hamsters was significantly reduced by pretreatment with 1 or 10 mg of mouse MAb EH3015, which belongs to the IgG1 family (12). In the present study, complete protection was detected only in 3 of 12 hamsters pretreated with 5 mg of XEhI-20, although a reduction of the mean abscess size was observed in all groups pretreated with one of the three adher-

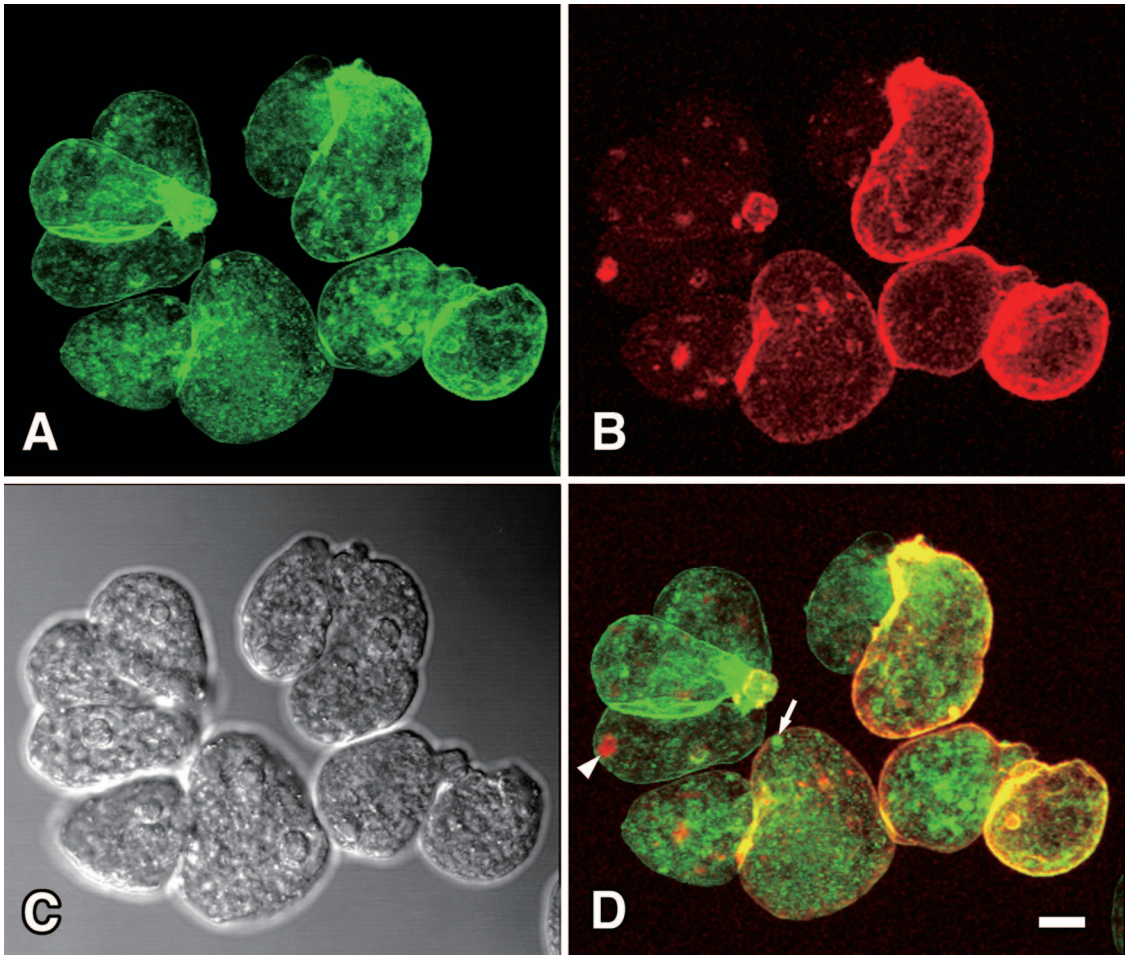

FIG. 5. Localization of Igl1 and Igl2 on trophozoites of *E. histolytica* HM-1:IMSS observed by confocal laser scanning microscopy. Fixed trophozoites were stained with Alexa Fluor 488-labeled XEhI-20, specific for Igl1 (green) (A), and Alexa Fluor 594-labeled XEhI-B5, specific for Igl2 (red) (B). A differential interference contrast microscopy image is shown in panel C. A merged image of panels A and B is shown in panel D. The arrow and arrowhead indicate the individual localization patterns of Igl1 and Igl2, respectively. The bar indicates 10 μm.

ence-inhibiting human MAbs to Igls. The difference in protective activity between the mouse and human MAbs may depend on differences of subtype and avidity for hamster complement and effector cells. In a mouse model, the mechanisms through

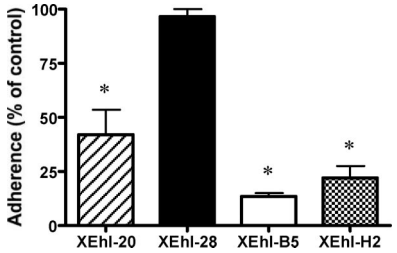

FIG. 6. Effects of human MAbs on adherence between *E. histolytica* and CHO cells. Trophozoites (10^4) were pretreated with 10 μg of MAb. The rate of adherence is expressed as a percentage of the adherence seen with PBS-treated controls. The results are presented as means ± standard deviations of data from four experiments. Asterisks indicate *P* values of <0.0001 (for comparison with the PBS control), 0.0029 (for XEhI-20 versus XEhI-B5), 0.0212 (for XEhI-20 versus XEhI-H2), and 0.0284 (for XEhI-B5 versus XEhI-H2).

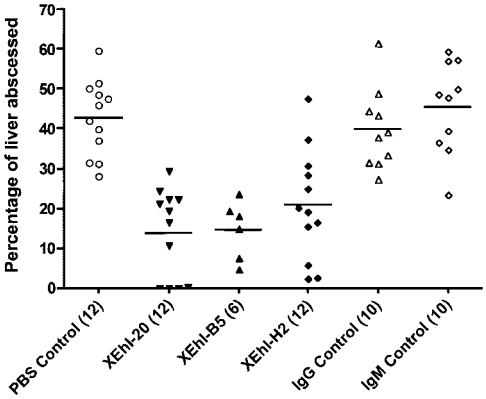

FIG. 7. Effects of human MAbs on amebic liver abscess formation in hamsters. Each hamster received an intraperitoneal injection of 0.5 ml of PBS containing 5 mg of human MAb 24 h before intrahepatic inoculation with *E. histolytica* trophozoites. Control hamsters received 0.5 ml of PBS only or 0.5 ml of PBS containing 5 mg of human polyclonal IgG or IgM. Abscess size is expressed as a percentage of the size of the abscessed liver. Horizontal bars indicate the mean values for each group. Numbers in parentheses indicate the number of hamsters in each group. XEhI-20 versus IgG control, $P < 0.0001$; XEhI-B5 versus IgG control, $P < 0.0001$; XEhI-H2 versus IgM control, $P = 0.0003$.

which human *E. histolytica*-specific antibody may provide protection include the inhibition of amebic adherence to target cells, antibody-dependent cell-mediated cytotoxicity, and complement activation and lysis of trophozoites (43). It is probable that rodent Fc receptors have avidities similar to those of human immunoglobulin. However, the subtypes of MAbs described in this paper are IgG2 and IgM. Therefore, it is likely that hamster Fc receptors do not bind to the human MAbs, although complement activation may occur.

In the gene analysis of the heavy-chain variable region, the closest germ lines for the V and D segments of the four human MAbs were different, and an IgBLAST search for these MAbs did not identify any identical immunoglobulin genes in the database. XenoMouse mice contain approximately 2 Mb of the human immunoglobulin heavy and κ light chain loci, which functionally recapitulate the human humoral immune system (32). Analyses of human antibody transcripts from XenoMouse spleens and lymph nodes demonstrate that V, D, and J gene utilization patterns in nonimmunized animals are nearly identical to the gene utilization patterns reported for humans with extensive antigenic histories (16). In our previous study, analyses of heavy-chain genes for anti-Hgl human MAb Fab fragments revealed that all the closest germ lines for V segments belong to the VH3 family (52). In the present study, all the IgG2 MAbs also belonged to the VH3 family, and this finding appears to agree well with the proposal that VH3 antibodies are important for defense against a variety of viruses (2, 25) and bacteria (1, 44).

A high prevalence of *E. histolytica* infection in patients with human immunodeficiency virus (HIV) infection and AIDS in Japan and Taiwan has been reported recently (23, 24, 33). Higher rates of seropositivity for *E. histolytica* in HIV-infected patients than in non-HIV-infected patients with gastrointestinal symptoms in China have also been demonstrated previously (7). However, anti-*E. histolytica* antibodies have occasionally not been detected in AIDS patients with invasive amebiasis (34, 41). Extraintestinal amebiasis, and especially amebic liver abscess, is the major cause of high mortality from amebiasis, and therefore, passive immunization with the human MAbs reported in the present study may be applicable in immunoprophylaxis to prevent the development of invasive amebiasis in immunodeficient patients. The fully human MAbs to Igls may provide a new strategy for the control of amebiasis.

ACKNOWLEDGMENTS

We thank Y. Okada for his help with flow cytometry analysis.

This work was supported by a grant-in-aid for scientific research from the Japanese Society for the Promotion of Science and a grant from the Ministry of Health, Labor and Welfare of Japan (to H.T.).

REFERENCES

1. **Abadi, J., J. Friedman, R. A. Mageed, R. Jefferis, M. C. Rodriguez-Barradas, and L. Pirofski.** 1998. Human antibodies elicited by a pneumococcal vaccine express idiotypic determinants indicative of V(H)3 gene segment usage. J. Infect. Dis. **178:**707–716.
2. **Andris, J. S., P. H. Ehrlich, L. Ostberg, and J. D. Capra.** 1992. Probing the human antibody repertoire to exogenous antigens. Characterization of the H and L chain V region gene segments from anti-hepatitis B virus antibodies. J. Immunol. **149:**4053–4059.
3. **Barbas, C. F., III, E. Bjorling, F. Chiodi, N. Dunlop, D. Cababa, T. M. Jones, S. L. Zebedee, M. A. Persson, P. L. Nara, E. Norrby, et al.** 1992. Recombinant human Fab fragments neutralize human type 1 immunodeficiency virus in vitro. Proc. Natl. Acad. Sci. USA **89:**9339–9343.
4. **Bekker, P. J., D. L. Holloway, A. S. Rasmussen, R. Murphy, S. W. Martin, P. T. Leese, G. B. Holmes, C. R. Dunstan, and A. M. DePaoli.** 2004. A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J. Bone Miner. Res. **19:** 1059–1066.
5. **Bird, R. E., K. D. Hardman, J. W. Jacobson, S. Johnson, B. M. Kaufman, S. M. Lee, T. Lee, S. H. Pope, G. S. Riordan, and M. Whitlow.** 1988. Single-chain antigen-binding proteins. Science **242:**423–426.
6. **Braga, L. L., H. Ninomiya, J. J. McCoy, S. Eacker, T. Wiedmer, C. Pham, S. Wood, P. J. Sims, and W. A. Petri, Jr.** 1992. Inhibition of the complement membrane attack complex by the galactose-specific adhesion of Entamoeba histolytica. J. Clin. Investig. **90:**1131–1137.
7. **Chen, Y., Y. Zhang, B. Yang, T. Qi, H. Lu, X. Cheng, and H. Tachibana.** 2007. Seroprevalence of Entamoeba histolytica infection in HIV-infected patients in China. Am. J. Trop. Med. Hyg. **77:**825–828.
8. **Cheng, X., K. Watanabe, S. Ihara, M. Takekoshi, and H. Tachibana.** 2000. Bacterial expression of a human monoclonal antibody that inhibits in vitro adherence of Entamoeba histolytica trophozoites. Arch. Med. Res. **31:**S311–S312.
9. **Cheng, X. J., M. A. Hughes, C. D. Huston, B. Loftus, C. A. Gilchrist, L. A. Lockhart, S. Ghosh, V. Miller-Sims, B. J. Mann, W. A. Petri, Jr., and H. Tachibana.** 2001. Intermediate subunit of the Gal/GalNAc lectin of *Entamoeba histolytica* is a member of a gene family containing multiple CXXC sequence motifs. Infect. Immun. **69:**5892–5898.
10. **Cheng, X. J., Y. Kaneda, and H. Tachibana.** 1997. A monoclonal antibody against the 150-kDa surface antigen of Entamoeba histolytica inhibits adherence and cytotoxicity to mammalian cells. Med. Sci. Res. **25:**159–161.
11. **Cheng, X. J., and H. Tachibana.** 2001. Protection of hamsters from amebic liver abscess formation by immunization with the 150- and 170-kDa surface antigens of Entamoeba histolytica. Parasitol. Res. **87:**126–130.
12. **Cheng, X. J., H. Tachibana, and Y. Kaneda.** 1999. Protection of hamsters from amebic liver abscess formation by a monoclonal antibody to a 150-kDa surface lectin of Entamoeba histolytica. Parasitol. Res. **85:**78–80.
13. **Cheng, X. J., H. Tsukamoto, Y. Kaneda, and H. Tachibana.** 1998. Identification of the 150-kDa surface antigen of Entamoeba histolytica as a galactose- and N-acetyl-D-galactosamine-inhibitable lectin. Parasitol. Res. **84:** 632–639.
14. **Cohen, B. D., D. A. Baker, C. Soderstrom, G. Tkalcevic, A. M. Rossi, P. E. Miller, M. W. Tengowski, F. Wang, A. Gualberto, J. S. Beebe, and J. D. Moyer.** 2005. Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. Clin. Cancer Res. **11:**2063–2073.
15. **Diamond, L. S., D. R. Harlow, and C. C. Cunnick.** 1978. A new medium for the axenic cultivation of Entamoeba histolytica and other Entamoeba. Trans. R. Soc. Trop. Med. Hyg. **72:**431–432.
16. **Gallo, M. L., V. E. Ivanov, A. Jakobovits, and C. G. Davis.** 2000. The human immunoglobulin loci introduced into mice: V (D) and J gene segment usage similar to that of adult humans. Eur. J. Immunol. **30:**534–540.
17. **Green, L. L.** 1999. Antibody engineering via genetic engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. J. Immunol. Methods **231:**11–23.
18. **Green, L. L., M. C. Hardy, C. E. Maynard-Currie, H. Tsuda, D. M. Louie, M. J. Mendez, H. Abderrahim, M. Noguchi, D. H. Smith, Y. Zeng, et al.** 1994. Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. Nat. Genet. **7:**13–21.
19. **Guo, X., E. Houpt, and W. A. Petri, Jr.** 2007. Crosstalk at the initial encounter: interplay between host defense and ameba survival strategies. Curr. Opin. Immunol. **19:**376–384.
20. **Haque, R., I. M. Ali, R. B. Sack, B. M. Farr, G. Ramakrishnan, and W. A. Petri, Jr.** 2001. Amebiasis and mucosal IgA antibody against the Entamoeba histolytica adherence lectin in Bangladeshi children. J. Infect. Dis. **183:**1787–1793.
21. **Haque, R., D. Mondal, P. Duggal, M. Kabir, S. Roy, B. M. Farr, R. B. Sack, and W. A. Petri, Jr.** 2006. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. Infect. Immun. **74:**904–909.
22. **Houpt, E., L. Barroso, L. Lockhart, R. Wright, C. Cramer, D. Lyerly, and W. A. Petri.** 2004. Prevention of intestinal amebiasis by vaccination with the Entamoeba histolytica Gal/GalNac lectin. Vaccine **22:**611–617.
23. **Hung, C. C., P. J. Chen, S. M. Hsieh, J. M. Wong, C. T. Fang, S. C. Chang, and M. Y. Chen.** 1999. Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. AIDS **13:**2421–2428.
24. **Hung, C. C., H. Y. Deng, W. H. Hsiao, S. M. Hsieh, C. F. Hsiao, M. Y. Chen, S. C. Chang, and K. E. Su.** 2005. Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch. Intern. Med. **165:**409–415.
25. **Ikematsu, H., N. Harindranath, Y. Ueki, A. L. Notkins, and P. Casali.** 1993. Clonal analysis of a human antibody response. II. Sequences of the VH genes of human IgM, IgG, and IgA to rabies virus reveal preferential utilization of VHIII segments and somatic hypermutation. J. Immunol. **150:** 1325–1337.
26. **Ivory, C. P., and K. Chadee.** 2007. Activation of dendritic cells by the

Gal-lectin of Entamoeba histolytica drives Th1 responses in vitro and in vivo. Eur. J. Immunol. **37:**385–394.
27. **Ivory, C. P., and K. Chadee.** 2007. Intranasal immunization with Gal-inhibitable lectin plus an adjuvant of CpG oligodeoxynucleotides protects against *Entamoeba histolytica* challenge. Infect. Immun. **75:**4917–4922.
28. **Jakobovits, A., R. G. Amado, X. Yang, L. Roskos, and G. Schwab.** 2007. From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. Nat. Biotechnol. **25:**1134–1143.
29. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
30. **Lotter, H., H. Russmann, J. Heesemann, and E. Tannich.** 2008. Attenuated recombinant Yersinia as live oral vaccine carrier to protect against amoebiasis. Int. J. Med. Microbiol. **298:**79–86.
31. **Marinets, A., T. Zhang, N. Guillen, P. Gounon, B. Bohle, U. Vollmann, O. Scheiner, G. Wiedermann, S. L. Stanley, and M. Duchene.** 1997. Protection against invasive amebiasis by a single monoclonal antibody directed against a lipophosphoglycan antigen localized on the surface of Entamoeba histolytica. J. Exp. Med. **186:**1557–1565.
32. **Mendez, M. J., L. L. Green, J. R. Corvalan, X. C. Jia, C. E. Maynard-Currie, X. D. Yang, M. L. Gallo, D. M. Louie, D. V. Lee, K. L. Erickson, J. Luna, C. M. Roy, H. Abderrahim, F. Kirschenbaum, M. Noguchi, D. H. Smith, A. Fukushima, J. F. Hales, S. Klapholz, M. H. Finer, C. G. Davis, K. M. Zsebo, and A. Jakobovits.** 1997. Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. Nat. Genet. **15:**146–156.
33. **Ohnishi, K., Y. Kato, A. Imamura, M. Fukayama, T. Tsunoda, Y. Sakaue, M. Sakamoto, and H. Sagara.** 2004. Present characteristics of symptomatic Entamoeba histolytica infection in the big cities of Japan. Epidemiol. Infect. **132:**57–60.
34. **Ohnishi, K., M. Murata, and E. Okuzawa.** 1994. Symptomatic amebic colitis in a Japanese homosexual AIDS patient. Intern. Med. **33:**120–122.
35. **Okada, M., C. D. Huston, B. J. Mann, W. A. Petri, Jr., K. Kita, and T. Nozaki.** 2005. Proteomic analysis of phagocytosis in the enteric protozoan parasite *Entamoeba histolytica*. Eukaryot. Cell **4:**827–831.
36. **Okada, M., C. D. Huston, M. Oue, B. J. Mann, W. A. Petri, Jr., K. Kita, and T. Nozaki.** 2006. Kinetics and strain variation of phagosome proteins of Entamoeba histolytica by proteomic analysis. Mol. Biochem. Parasitol. **145:**171–183.
37. **Ostendorf, T., C. R. van Roeyen, J. D. Peterson, U. Kunter, F. Eitner, A. J. Hamad, G. Chan, X. C. Jia, J. Macaluso, G. Gazit-Bornstein, B. A. Keyt, H. S. Lichenstein, W. J. LaRochelle, and J. Floege.** 2003. A fully human monoclonal antibody (CR002) identifies PDGF-D as a novel mediator of mesangioproliferative glomerulonephritis. J. Am. Soc. Nephrol. **14:**2237–2247.
38. **Petri, W. A., Jr., M. D. Chapman, T. Snodgrass, B. J. Mann, J. Broman, and J. I. Ravdin.** 1989. Subunit structure of the galactose and N-acetyl-D-galactosamine-inhibitable adherence lectin of Entamoeba histolytica. J. Biol. Chem. **264:**3007–3012.
39. **Petri, W. A., Jr., R. Haque, and B. J. Mann.** 2002. The bittersweet interface of parasite and host: lectin-carbohydrate interactions during human invasion by the parasite Entamoeba histolytica. Annu. Rev. Microbiol. **56:**39–64.
40. **Saito-Nakano, Y., B. N. Mitra, K. Nakada-Tsukui, D. Sato, and T. Nozaki.** 2007. Two Rab7 isotypes, EhRab7A and EhRab7B, play distinct roles in biogenesis of lysosomes and phagosomes in the enteric protozoan parasite Entamoeba histolytica. Cell. Microbiol. **9:**1796–1808.
41. **Sanuki, J., E. Okuzawa, E. Imai, S. Kobayashi, G. Masuda, and T. Takeuchi.** 1995. Concurrent infection of homosexual men with human immunodeficiency virus and Entamoeba histolytica in Japan: serodiagnosis of amebic infection. Jpn. J. Parasitol. **44:**421–425.
42. **Seguin, R., B. J. Mann, K. Keller, and K. Chadee.** 1997. The tumor necrosis factor alpha-stimulating region of galactose-inhibitable lectin of *Entamoeba histolytica* activates gamma interferon-primed macrophages for amebicidal activity mediated by nitric oxide. Infect. Immun. **65:**2522–2527.
43. **Seydel, K. B., K. L. Braun, T. Zhang, T. F. Jackson, and S. L. Stanley, Jr.** 1996. Protection against amebic liver abscess formation in the severe combined immunodeficient mouse by human anti-amebic antibodies. Am. J. Trop. Med. Hyg. **55:**330–332.
44. **Silverman, G. J., and A. H. Lucas.** 1991. Variable region diversity in human circulating antibodies specific for the capsular polysaccharide of Haemophilus influenzae type b. Preferential usage of two types of VH3 heavy chains. J. Clin. Investig. **88:**911–920.
45. **Tachibana, H., X. J. Cheng, S. Kobayashi, Y. Okada, J. Itoh, and T. Takeuchi.** 2007. Primary structure, expression and localization of two intermediate subunit lectins of Entamoeba dispar that contain multiple CXXC motifs. Parasitology **134:**1989–1999.
46. **Tachibana, H., X. J. Cheng, G. Masuda, N. Horiki, and T. Takeuchi.** 2004. Evaluation of recombinant fragments of *Entamoeba histolytica* Gal/GalNAc lectin intermediate subunit for serodiagnosis of amebiasis. J. Clin. Microbiol. **42:**1069–1074.
47. **Tachibana, H., X. J. Cheng, K. Watanabe, M. Takekoshi, F. Maeda, S. Aotsuka, Y. Kaneda, T. Takeuchi, and S. Ihara.** 1999. Preparation of recombinant human monoclonal antibody Fab fragments specific for *Entamoeba histolytica*. Clin. Diagn. Lab Immunol. **6:**383–387.
48. **Tachibana, H., S. Kobayashi, X. J. Cheng, and E. Hiwatashi.** 1997. Differentiation of Entamoeba histolytica from E. dispar facilitated by monoclonal antibodies against a 150-kDa surface antigen. Parasitol. Res. **83:**435–439.
49. **Tachibana, H., S. Kobayashi, Y. Kaneda, T. Takeuchi, and T. Fujiwara.** 1997. Preparation of a monoclonal antibody specific for *Entamoeba dispar* and its ability to distinguish *E. dispar* from *E. histolytica*. Clin. Diagn. Lab Immunol. **4:**409–414.
50. **Tachibana, H., S. Kobayashi, Y. Kato, K. Nagakura, Y. Kaneda, and T. Takeuchi.** 1990. Identification of a pathogenic isolate-specific 30,000-M_r antigen of *Entamoeba histolytica* by using a monoclonal antibody. Infect. Immun. **58:**955–960.
51. **Tachibana, H., M. Takekoshi, X. J. Cheng, F. Maeda, S. Aotsuka, and S. Ihara.** 1999. Bacterial expression of a neutralizing mouse monoclonal antibody Fab fragment to a 150-kilodalton surface antigen of Entamoeba histolytica. Am. J. Trop. Med. Hyg. **60:**35–40.
52. **Tachibana, H., K. Watanabe, X. J. Cheng, H. Tsukamoto, Y. Kaneda, T. Takeuchi, S. Ihara, and W. A. Petri, Jr.** 2003. VH3 gene usage in neutralizing human antibodies specific for the *Entamoeba histolytica* Gal/GalNAc lectin heavy subunit. Infect. Immun. **71:**4313–4319.
53. **Tomizuka, K., H. Yoshida, H. Uejima, H. Kugoh, K. Sato, A. Ohguma, M. Hayasaka, K. Hanaoka, M. Oshimura, and I. Ishida.** 1997. Functional expression and germline transmission of a human chromosome fragment in chimaeric mice. Nat. Genet. **16:**133–143.
54. **Walsh, J. A.** 1986. Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. Rev. Infect. Dis. **8:**228–238.
55. **Zhang, T., P. R. Cieslak, L. Foster, C. Kunz-Jenkins, and S. L. Stanley, Jr.** 1994. Antibodies to the serine rich Entamoeba histolytica protein (SREHP) prevent amoebic liver abscess in severe combined immunodeficient (SCID) mice. Parasite Immunol. **16:**225–230.

Editor: W. A. Petri, Jr.