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Therapeutic Antibody Targeting of CD97 in Experimental Arthritis: the Role of Antigen Expression, Shedding, and Internalization on the Pharmacokinetics of Anti-CD97 Monoclonal Antibody 1B2

Dorien M. de Groot,* Gerard Vogel,* John Dulos,* Leonie Teeuwen,* Karin Stebbins,‡ Jörg Hamann,‡ Bronwyn M. Owens,‡ Hans van Eenennaam,* Ebo Bos,* and Annemieke M. Boots2*

CD97 is a member of the EGF-TM7 family of adhesion class receptors, with a proposed role in inflammatory cell recruitment. Neutralization of murine CD97 with the anti-mCD97 mAb 1B2 was efficacious in prevention of murine collagen-induced arthritis, a model with features resembling rheumatoid arthritis. Here, the therapeutic potential of neutralizing CD97 in arthritis was studied with emphasis on the 1B2 pharmacokinetics. Mice with established arthritis were treated with anti-mCD97 or anti-TNF-α serum. Ab pharmacokinetics and biodistribution were studied in diseased and nondiseased mice using labeled 1B2. The impact of CD97 expression on Ab pharmacokinetics was studied using CD97 knockout mice. Treatment with 1B2 showed an efficacy comparable to anti-TNF-α treatment. Pharmacokinetic analysis of 1B2 in wild-type and CD97 knockout mice indicated a dose-dependent Ab clearance, due to specific interaction with CD97. Biodistribution studies showed accumulation of 1B2 in spleen and lung. In vitro studies using murine splenocytes revealed that CD97 when bound to Ab was internalized. Moreover, soluble CD97 was detected in the supernatant, suggesting Ag shedding. Finally, in arthritic mice, higher levels of soluble CD97 were found and 1B2 treatment led to specific targeting of inflamed paws, resulting in a higher clearance rate of 1B2 in arthritic mice than in wild-type mice. In conclusion, our data support a therapeutic value of CD97 neutralization in experimental arthritis. The pharmacokinetic profile of the 1B2 Ab illustrates the complexity of Ab elimination from an organism and stresses the importance of understanding Ag-Ab interactions when developing therapeutic mAbs. The Journal of Immunology, 2009, 183: 4127–4134.

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whereas in the case of excess levels of mAb, clearance will be similar to endogenous IgG, largely determined by the reticuloendothelial system (20).

In this study, the therapeutic potential of CD97 neutralization in experimental arthritis was examined using a design in which mice with established disease were treated with two different anti-mCD97 mAbs or with anti-TNF-α serum for comparison. Furthermore, the pharmacokinetics of the most effective anti-mCD97 Ab in both nondiseased and diseased mice was characterized. Our data underscore the importance of a careful examination of the target Ag when developing therapeutic mAbs.

Materials and Methods

Animals and Abs

Male DBA/1J mice (Bomholtgard), female BALB/c mice, male C57BL/6 mice (Charles River Laboratories), and male CD97 knockout mice (C57BL/6j background) (15) (Academic Medical Center, Amsterdam, The Netherlands) were group-housed under controlled conditions with ad libitum access to water and standard pelleted food. All animal procedures and experiments were approved by the animal ethical committee and were performed under the guidelines of the Dutch law concerning animal welfare.

Hamster anti-mouse CD97 Abs 1B2 (directed against EGF domain 1), 1A2 (directed against EGF domain 2), and 1C5 (directed against EGF domain 3) were used (6, 13) (Fig. 1A). Hamster anti-human EMR3 Ab 3D7 was used as isotype control (21). An ELISA employing rabbit anti-hamster IgG (SouthernBiotech) and goat anti-hamster IgG-HRP (Serotec) was used as isotype control (21). An ELISA employing rabbit anti-hamster IgG (SouthernBiotech) and goat anti-hamster IgG-HRP (Serotec) was used to detect hamster IgGs in serum. To neutralize TNF-α or IL-1β in CIA, rabbit polyclonal anti-TNF-α or anti-IL-1β serum and control rabbit IgG were used as described (22, 23).

Therapeutic CIA model

The murine therapeutic CIA model was essentially performed as described (24, 25). In brief, male DBA/1J mice were immunized at the base of the tail with 100 μg of bovine type II collagen in CFA enriched with 2 mg/ml Mycobacterium tuberculosis H37Ra. Three weeks after immunization, the animals were given an i.p. booster injection of 100 μg of bovine type II collagen dissolved in saline. After disease onset, animals with an arthritis score ranging from 0.25 to 1.25 were divided into separate groups of 11–12 mice so that the mean arthritis score of all treatment groups was comparable at the start of treatment (day 0). Four independent experiments were done in which Abs (25 mg/kg unless mentioned otherwise) or PBS were given to arthritic animals via an i.p. injection, thrice a week, for a period of 21 days. As a positive control for suppression of arthritis, animals were treated p.o. with 1.5 mg/kg prednisolone suspended in 0.5% gelatin and 0.5% mannitol in water each day, or i.p. with 1.5 mg/kg anti-IL-1β Abs thrice a week. All experimental treatments were conducted in a blinded fashion.

The clinical severity of arthritis (arthritis score) was graded (a scale of 0–2 for each paw) and scored as described (22, 26). To assess the effects of Ab treatments, the area under the curve of mean arthritis score of each animal with baseline correction (subtracting baseline area under the curve of arthritis score on day 0) was used. At the end of the experiments, knee and ankle joints were used for radiological or histological analyses as described (22). Bone destruction was scored on a scale of 0–5 per joint, ranging from no damage to complete destruction (23), and per animal a cumulative score of both hind paws and knees was calculated. From H&E-stained sections, articular cartilage destruction and inflammatory infiltrate in the right knee joint were scored on a scale of 0–3. To compare and combine data from independent experiments, data per experiment were normalized to the average value of the PBS-treated group.

**FIGURE 1.** Therapeutic effect of anti-mCD97 or anti-TNF-α Abs on established collagen-induced arthritis in mice. A, Schematic structure of the largest isoform mCD97(EGF1,2,X,3,4), possessing four EGF domains, interrupted by a domain of unknown origin (X). Indicated are the binding sites of the mAbs 1B2, 1A2, and 1C5 within EGF domain 1, EGF domain 2, and EGF domain 3, respectively. Next to full-length CD97, smaller isoforms exist that are designated mCD97(EGF1,2,4) and mCD97(EGF1,2,3,4) (see Introduction). While 1B2 and 1A2 recognize all mCD97 isoforms, binding of 1C5 is restricted to the two larger isoforms. B, The effect of neutralizing mCD97 or TNF-α in CIA was examined in three or two independent experiments, respectively. B and C show representative examples of a time course profile of mean arthritis scores ± SEM of 11–12 animals. To compare and aggregate the data of different experiments, the areas under the curve were normalized to PBS treatment (D). The results are means ± SEM of the number of animals indicated in the bars, **, p < 0.01 and ****, p < 0.0001 significantly different from control group (3D7, rabbit IgG, vehicle) using ANOVA followed by Fisher’s least significant difference test.
Biotinylation and radiolabeling of Abs

1B2, 1A2, and 3D7 Abs were biotinylated using EZ-Link NHS-biotin according to the manufacturer’s instructions (Pierce Biotechnology). 1B2 and 3D7 Abs were iodinated using precoated iodination tubes (Pierce Biotechnology). Per labeling reaction, 250 µg of each Abs was used. As a control, the immunoreactivity of 125I-labeled and nonlabeled Abs was compared. For this, ARHO cells stably expressing mCD97 were incubated for 1 h at 37°C with a concentration series (3–0.01 µg/ml) of 125I-labeled or nonlabeled 1B2 or 3D7. After washing with PBS, 0.1% Tween 20 (PBST), cells were incubated for 1 h with 1/1000 diluted goat anti-hamster IgG-HRP (ITK Diagnostics). Following washing with PBST and addition of tetramethylbenzidine, reactions were stopped with H2SO4 and the absorbance was read at 450 nm.

Generation of recombinant mouse CD97 protein

The pcdNA3.1 (+)neo vector containing the mCD97(EGF1,2,4)-mlgG2b construct was generated as described (10). For generation of recombinant protein, transient transfections were performed using the FreeStyle 293 Expression System (Invitrogen) according to the manufacturer’s protocol. Recombinant protein-containing supernatants were concentrated and passed over a Montage PROSEP-A column (Millipore). Protein was eluted from the column in PBS (pH 9.0) and concentrated using a 50-KDa Amicon filter (Millipore).

Pharmacokinetics and biodistribution

Pharmacokinetic characteristics of different 1B2 concentrations were determined in male DBA/1J mice (wild type; wt); healthy control mice, CD97 knockout (KO) mice, or arthritic mice. In all experiments, two or three mice per time point were used, and Abs were injected i.p. or i.v. After injection of 0.1 or 0.04 mg/kg 125I-radioabeled 1B2 or 3D7, supplemented with 0, 6.25, 12.5, or 25 mg/kg nonlabeled Ab, blood was collected at various time points. From heparinized blood samples, 0.1 ml of plasma or blood was precipitated using 10% TCA, and radioactivity of the pellet was determined using a gamma counter (Cobra; Packard). Plasma concentrations of 1B2 or 3D7 were calculated by multiplying the measured radioactivity of the pellet with the specific radioactivity of the injected Abs, and then divided over the amount of injected nonlabeled Abs. Clearance rates were calculated using WinNonlin software (Pharsight). Since only a limited number of samples in time were collected, the calculated clearance rates should be considered as rough estimates.

The biodistribution of 1B2 and 3D7 was determined in male DBA/1J and in arthritic mice. After i.p. injection with 125I-radioabeled Ab, blood was collected and mice were perfused with 0.9% NaCl for 5 min. Next, various organs (as indicated in Fig. 3C) were collected and weighed. Radioactivity of the organs was measured using a gamma counter (Cobra) and expressed as the percentage of injected dose per gram tissue. To establish CD97-specific mAb accumulation in arthritic vs nonarthritic paws, the radioactivity of paws was measured at 72 h after injection of radiolabeled 1B2 or isotype control 3D7 and expressed as radioactivity of 100 mg of tissue/radioactivity of 100 µl of blood. Subsequently, the average value of the isotype control was subtracted from the values of 1B2.

Copy number, internalization, and shedding of mCD97

Isolated mouse (BALB/c) splenocytes were used to determine copy number, internalization, and shedding of mCD97. For each experiment, 5 × 10^7 cells were seeded per well in multiscreen plates (MAHVNI4510; Millipore). To determine the copy number and shedding of CD97, cells were incubated for 2 h at 4°C or 37°C with 125I-radioabeled 1B2 or 3D7 in culture medium. For internalization studies, cells were incubated for 2 h with 125I-radioabeled 1B2 or 3D7 in medium at 37°C, followed by a 30-min incubation at 4°C in PBS or widely used low pH buffer (0.1 M glycine, 4 g/L NaCl (pH 2.0)) to strip off membrane-bound Ab (27). Before this experiment, it was established that the acidic elution buffer was very effective in acidic elution buffer was very effective in eluting 1B2 from bound mCD97 protein using a Biacore assay (data not shown). Finally, after washing three times with PBS using vacuum filtration, the remaining radioactivity of the cells on the filter was determined using a gamma counter (Cobra). The CD97 copy number per cell was calculated from this radioactive count and corrected for the efficiency of the gamma counter (∗100/60) and specific activity of the radiolabeled Abs (counts/specific activity), followed by a conversion from weight to number of moles (divided by molecular mass of Ab) and molecules (times Avogadro constant) per cell (divided by 5 × 10^7). Next, shedding of CD97 was examined using this method. For this, splenocytes were saturated for 2 h at 4°C with 125I-radioabeled or biotinylated 1B2 or 3D7 in medium, washed three times with ice-cold PBS, and incubated at 37°C or 4°C, after which supernatants were collected at different time points. Radioactivity of the supernatant was counted (gamma counter; Cobra) or levels of sCD97 bound to 1B2 were measured using an ELISA and expressed as the OD at 450 nm.

ELISA

sCD97 in serum was measured using 1B2 (capture Ab), 1A2-biotin (detection Ab), and streptavidin-HRP. sCD97 bound to 1B2-biotin in serum samples from the shedding experiments was detected using IC5 (capture Ab) and streptavidin-HRP. Levels of 1B2 or 3D7 in sera of CIA mice were measured using rabbit anti-hamster IgG (capture Ab) and goat anti-hamster IgG-HRP (detection Ab). Plates were coated overnight with 1 µg/ml capture Ab in PBS (1B2 and IC5) or coat buffer (0.2 M NaCO3/NaHCO3 (pH 9.6); Pierce) (anti-hamster IgG). Blocking was performed in 5% BCS (1B2 and 1C5) or BSA (anti-hamster IgG) in PBST, after which samples were added to the wells. Subsequently, detection Ab, streptavidin-HRP, and tetramethylbenzidine were added. Reactions were stopped with H2SO4, and the absorbance read at 450 nm. All incubations were performed for 1 h at room temperature, and after each incubation step samples were washed with PBST. Semi-quantitative levels of sCD97 were calculated from a standard of recombinant mouse sCD97(EGF1,2,4)-mFc that was included on each plate. The data were statistically analyzed using ANOVA followed by Dunnet’s multiple comparison test. Hamster IgG levels in sera from CIA mice were calculated from a standard of 1B2 or 3D7 Ab. No standard was available for the complex of sCD97 bound to 1B2-biotin, and therefore qualitative levels were expressed as absorbance levels.

Results

Neutralization of mCD97 is equally effective as anti-TNF-α in treatment of established CIA

Previously, it was shown that CD97 neutralization with anti-mCD97 (mAb 1B2) increases resistance to CIA induction (16). To confirm and extend these results, two different anti-mCD97 mAbs were evaluated for suppression of an ongoing arthritis. In three independent experiments, treatment with 25 mg/kg 1B2 three times a week for 3 wk significantly reduced the mean arthritis score by ~60% (p < 0.0001) (Fig. 1B–D). In animals treated with 12.5 mg/kg 1B2 no significant effect on arthritis development was observed (Fig. 1D). Treatment with mAb 1C5 (recognizing the EGF3 domain of mCD97) resulted in a 40% decrease in mean arthritis score, which did not reach significance (p = 0.057) (Fig. 1, B and D). Therefore, mAb 1B2 was used for further studies.

Next, the efficacy of 1B2 was compared with anti-TNF-α treatment. In two independent experiments, treatment with anti-TNF-α antiserum resulted in a significant decrease in arthritis score by ~60% (p < 0.0001) (Fig. 1, C and D). This reduction was similar to the efficacy of 1B2 treatment (Fig. 1, C and D). The effects of 25 mg/kg 1B2 and anti-TNF-α on parameters relevant for arthritis pathology, such as bone erosion, cartilage destruction, and inflammatory infiltrate in affected joints, were examined. Radiological analysis of bone erosion in knee and ankle joints revealed a significant amelioration of bone damage by 1B2 or anti-TNF-α treatment (Fig. 2A). Notably, 1B2 or anti-TNF-α treatment did not significantly affect inflammatory cell infiltration or prevent cartilage destruction (Fig. 2, B and C). Thus, neutralization of mCD97 compares well to anti-TNF-α treatment in suppression of established CIA and suggests that interactions of mCD97 with endogenous ligands play a role in arthritis.

Soluble CD97 and neutralization by 1B2

Elevated levels of sCD97 have been found in synovial fluid of patients with RA (5, 17). Therefore, levels of sCD97 in sera from arthritic mice were examined using a sandwich-type ELISA, with an Ab pair detecting all CD97 isoforms. Sera from mice receiving PBS, 3D7, or 1B2 (25 and 12.5 mg/kg) and from mice without clinical signs after collagen immunization (nondiseased) were assayed. Higher levels of sCD97 were detected in diseased mice (PBS or 3D7 treated) vs nondiseased mice (Fig. 2D, left panel). Sera from 1B2-treated mice showed significantly less (non-Ab bound) sCD97. The level of detection was inversely correlated.
with the mAb dose, suggesting that sCD97 is bound to the 1B2 Ab. In line with this, levels were lower in mice treated with the highest mAb dose (Fig. 2D, right panel). Additionally, in anti-IL-1β-treated mice with reduced signs of arthritis, levels of sCD97 were significantly higher compared with the 1B2-treated animals (data not shown), demonstrating that the lower levels of sCD97 measured in 1B2-treated mice are not directly resulting from reduced disease.

Dose-dependent clearance of 1B2 is due to a specific interaction with mCD97

To evaluate the therapeutic potential of anti-CD97 treatment in arthritis, more insight in the basic biology of mCD97 and its interaction with 1B2 was required. First, a limited pharmacokinetic experiment with 1B2 and its isotype control 3D7 in wt and CD97 KO mice was performed. After injection of 0.04 mg/kg radiolabeled Ab, 1B2 and 3D7 plasma levels were determined at 4 and 48 h. In wt animals the levels of 1B2 were much lower compared with those of 3D7 both at 4 and 48 h after injection, suggesting a faster clearance of 1B2 (Fig. 3A). In contrast, in CD97 KO animals plasma levels of 1B2 and 3D7 were comparable (Fig. 3A), indicating that the rapid clearance of 1B2 in wt animals is due to a specific interaction of 1B2 with mCD97.

The observed rapid disappearance of 1B2 prompted us to examine the pharmacokinetic profile of various concentrations of 1B2. For this purpose, mice were injected with radiolabeled 1B2 supplemented with various amounts of nonlabeled Ab, after which plasma levels of 1B2 were measured at various time points after injection (Fig. 3B). The binding properties of radiolabeled and nonlabeled Abs were comparable (data not shown). A dose-dependent clearance of 1B2 (Table I) was observed. Notably, whereas the pharmacokinetic profiles of 12.5 and 25 mg/kg 1B2 were similar to 3D7 (Fig. 3B), treatment with 12.5 mg/kg was not effective in CIA (Fig. 1D). This may be explained by increased Ag expression levels in diseased mice. In contrast, treatment with 25 mg/kg led to a significant suppression of disease (Fig. 1D), suggesting that at this dose full CD97 Ag saturation was achieved. This was further confirmed by measuring 1B2 and 3D7 levels in sera of Ab-treated CIA mice at the end of the experiment. Both 1B2 and 3D7 were detected in the serum at levels of ~170 and 290 μg/ml (data not shown).

In summary, the clearance rate of 1B2 is inversely correlated with the concentration of the Ab, and thus is dependent on the in vivo Ab-Ag ratio. The dosing used for the CIA experiments (25 mg/kg, three times per week for 3 wk) was high enough to saturate and neutralize the CD97 Ag and to achieve a therapeutic effect similar to that of anti-TNF-α.

Biodistribution of 1B2 in nondiseased mice

The 1B2 Ab is quickly removed from the circulation of wt mice (Fig. 3B). To find out whether 1B2 is targeted to specific organs, we examined the biodistribution of the Ab. Mice were thus injected with radiolabeled 1B2 or 3D7, and after 4 h the radioactivity in various organs was measured. Accumulation of 1B2, and not of the isotype control 3D7, was predominantly found in spleen and lung tissue (Fig. 3C), which is in accordance with reported mCD97 expression in spleen and lung (15). Of note, the spleen and lung tissues of animals receiving 1B2 (or 3D7) treatment, when subjected to histopathological examination, did not reveal any changes (data not shown).

Copy number, internalization, and shedding of mCD97

The elimination rate of 1B2 in vivo is determined by Ag expression levels (Ag copy number on cells), Ag internalization, which may be initiated following Ag-Ab interaction at the cell surface, and Ag shedding, which is the release of immunocomplexed Ag from the cell surface. To examine this in vitro, mouse splenocytes were used. First, to estimate the copy number of CD97, cells were saturated at 4°C with radiolabeled 1B2 or 3D7. Since the amount of

FIGURE 2. Effects of anti-mCD97 treatment on bone and cartilage destruction and infiltration. The effects of neutralizing mCD97 or TNF-α in CIA, on bone erosion (A), cartilage destruction (B), and inflammatory infiltrate in affected joints (C) were examined. To compare and aggregate the data of different experiments, data were normalized to PBS treatment per experiment. The results shown are mean scores ± SEM of the number of animals indicated in the bars. *, p < 0.05; **, p < 0.01; ****, p < 0.0001 significantly different from control group (3D7, rabbit IgG, vehicle) using ANOVA followed by Fisher’s least significant difference test. D, Levels of soluble mCD97 in sera of nondiseased or diseased CIA mice. Sera of mice were screened for sCD97 by ELISA using 1B2 and 1A2-biotin as capture and detection Abs, respectively. Bars represent the mean ± SEM of 11 animals, *, p < 0.05 and ***, p < 0.001, significant difference with nondiseased mice, and ###, p < 0.001, significant difference with 3D7-treated mice, using ANOVA followed by Dunnet’s multiple comparison test.
of bound 1B2 Ab is a measure for the amount of cell-surface CD97 Ag, the copy number of CD97 was calculated from the radioactive counts after 1B2 binding minus the background (radioactive counts after 3D7 incubation), and it was found to be \(2.5 \times 10^5\) (Fig. 4A).

Second, to examine possible internalization of CD97, cells were incubated for 2 h with radiolabeled 1B2 or 3D7 at 37°C, followed by a wash with PBS or a widely used low pH buffer to strip off all membrane-bound Abs (Fig. 4B). Subsequently, radioactivity of the cells was determined. The radioactive counts measured after low pH stripping indicated that a large part (69 ± 3%) of the CD97 Ag was internalized during the 2-h incubation period (Fig. 4A).

Third, the detection of significant levels of sCD97 in sera of CIA mice (Fig. 2D) prompted us to examine shedding of CD97 in vitro. For that purpose, mouse splenocytes were incubated for 2 h with radiolabeled 1B2 or 3D7 at either 4°C or 37°C. After incubation at 37°C the measured radioactivity was lower than at 4°C with 1B2 (Fig. 4A), implying shedding of CD97 during the 2-h incubation period at 37°C. To study this process in more detail, shedding of CD97 was examined in time. Splenocytes were saturated at 4°C with radiolabeled or biotinylated 1B2 or 3D7, after which cells were thoroughly washed and supplemented with fresh medium. At various time intervals the supernatant was collected and radioactivity was determined or levels of the complex of sCD97 bound to 1B2-biotin were measured using a specific ELISA. We detected a time-dependent release of radiolabeled 1B2 (Fig. 4C) or immunocomplexed CD97 (Fig. 4D) at 37°C, but not at 4°C, indicating shedding of CD97.

Thus, the data imply that CD97 Ag is both internalized and shed from murine splenocytes in vitro.

**Pharmacokinetic profile and biodistribution of 1B2 are influenced by the arthritic process in mice**

Significantly higher levels of sCD97 in sera of arthritic mice compared with nondiseased DBA-1 mice were detected (Fig. 2D). Since the pharmacokinetic characteristics of the 1B2 Ab are directly determined by its interaction with CD97 (Fig. 3), we examined the pharmacokinetics of 1B2 in arthritic mice compared with healthy control mice at 6.25 mg/kg. At various time points after Ab injection, blood was collected and Ab levels were determined. The clearance of 1B2 in arthritic mice (\(\sim 10\) ml/h/kg) was higher compared with control animals (6 ml/h/kg), whereas 3D7 was cleared at a similar rate as seen in control mice (Fig. 5A).

Furthermore, when studying the biodistribution of radiolabeled 1B2 in both control and CIA mice, accumulation of 1B2 was again detected in lung and spleen tissue (data not shown). Moreover, a significantly higher level of 1B2 was measured in paws of arthritic mice compared with control mice (6 ml/h/kg), whereas 3D7 was cleared at a similar rate as seen in control mice (Fig. 5B). The specific targeting of 1B2 Ab to the inflamed paws is probably due to higher expression levels of CD97 in the joint and/or “trapping” of 1B2 Ab by CD97-expressing cells. Thus, the diseased situation affects CD97 Ag expression levels, thereby directly affecting the pharmacokinetic properties of the (therapeutic) anti-CD97 mAb.

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Biologic therapies such as anti-TNF-\(\alpha\) treatment have greatly improved the management of chronic inflammatory diseases such as RA (28, 29). However, there is still a substantial group of RA patients that do not respond to or become resistant to therapy. Therefore, the need to identify new therapeutic targets for RA is high. Animal models provide a valuable tool to identify and evaluate novel targets. Here, a therapeutic CIA model was employed to test the potential of two different anti-mCD97 Abs. Treatment of arthritic animals with mAb 1B2 led to significant inhibition of arthritis development, whereas a trend toward suppression was seen with 1C5. The superior inhibition by 1B2 may be explained by the differential binding of 1B2 and 1C5 to various differentially expressed CD97 isoforms; 1B2 binds to EGF domain 1, present in all isoforms, whereas 1C5 is directed against EGF domain 3, present in only two of the three isoforms (6, 7). Since the ratios for the mouse isoforms mCD97(EGF1,2,4), (EGF1,2,3,4), and (EGF1,2,X,3,4) are \(\sim 45, 45, \text{ and } 10\%\) (our unpublished observations), treatment of mice with 1B2 would target \(\sim 50\%\) more target Ag compared with treatment with mAb 1C5, which is in line with a superior suppressive activity of 1B2 treatment. For comparison, in human isoforms hCD97(EGF1,2,5), (EGF1,2,3,5), and (EGF1,2,3,4,5) account for \(\sim 60, 30, \text{ and } 10\%\), respectively (our unpublished observations).

1B2 treatment in our CIA model equaled anti-TNF-\(\alpha\) treatment. The level of suppression was comparable to previous data using this anti-TNF-\(\alpha\) serum (22). Also, when examining bone destruction as an arthritis-associated destruction parameter, 1B2 and anti-TNF-\(\alpha\) treatments showed similar protective effects. The superior inhibition by 1B2 may be explained by the differential binding of 1B2 and 1C5 to various differentially expressed CD97 isoforms; 1B2 binds to EGF domain 1, present in all isoforms, whereas 1C5 is directed against EGF domain 3, present in only two of the three isoforms (6, 7). Since the ratios for the mouse isoforms mCD97(EGF1,2,4), (EGF1,2,3,4), and (EGF1,2,X,3,4) are \(\sim 45, 45, \text{ and } 10\%\) (our unpublished observations), treatment of mice with 1B2 would target \(\sim 50\%\) more target Ag compared with treatment with mAb 1C5, which is in line with a superior suppressive activity of 1B2 treatment. For comparison, in human isoforms hCD97(EGF1,2,5), (EGF1,2,3,5), and (EGF1,2,3,4,5) account for \(\sim 60, 30, \text{ and } 10\%\), respectively (our unpublished observations).

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To gain more insight in a possible therapeutic value of Ab-mediated CD97 neutralization in arthritis, we examined the pharmacokinetic characteristics of the 1B2 Ab. In general, effective Ab therapy requires favorable pharmacokinetics of the candidate mAb. For example, whereas TNF-\(\alpha\) blockade is clinically effective in RA, it has little benefit in treatment of systemic inflammatory
response syndrome despite identification of TNF-α as a key factor in its pathology. This difference in efficacy may be explained by differences in free levels of TNF-α between RA and systemic inflammatory response syndrome (30).

Ag-dependent clearance pathways are a well-known phenomenon for biologicals (20). In our studies, a dose-dependent clearance of 1B2 was found. In particular, lower doses of 1B2 were rapidly cleared from the circulation, due to accumulation of the Ab in the target organs lung and spleen, and binding to shed CD97 in plasma. In this respect, the production rate of sCD97 is a key factor in the elimination of 1B2 and thus in the therapeutic efficacy of the Ab. An estimated shedding rate of 2 x 10^6 molecules/cell/24 h (Fig. 4A) results in a daily supply of sCD97 in the order of 250–1000 pmol taking into account a total of 5 x 10^8 CD97+ expressing cells per animal. This is reflected in a sCD97 plasma concentration of 50 ng/ml in healthy animals and of 100 ng/ml in diseased animals. As a consequence, a higher clearance of 1B2 in diseased compared with control mice was observed. For comparison, a similar observation was made for mAb B43.13, directed against the CA125 Ag, which had a shorter residence time and faster clearance due to higher levels of circulating CA125 Ag in ovarian cancer patients (31).

Of note, a bolus injection of 1 nmol (6.25 mg/kg) Ab 1B2 is equivalent to 2–4 daily production portions of sCD97. Thus, it should be appreciated that at this initial Ag-Ab ratio the elimination rate of Ab increases after 30–50 h (Fig. 5A) because an increasing part of the Ab is being cleared as immunocomplex. It explains why high doses of 1B2 (i.e., 25 mg/kg thrice a week) are needed to block the CD97 Ag. Continuously high blood levels are apparently essential for achieving physiological efficacy.

The latter suggests that the mechanism underlying the therapeutic effect is cellular Ag blocking and/or elimination. Previously, it has been postulated that 1B2 exerts its beneficial effect by blockage of the interaction between CD97 and CD55, thereby affecting the recruitment of immune cells to the site of inflammation (13, 16). Evidence for the relevance of such an interaction in humans was found by the colocalization of CD55+ synovocytes and CD97+ macrophages in synovial tissue (17). A second putative mechanism of 1B2 efficacy in vivo was hypothesized recently based on evidence suggesting that 1B2 activates CD97, rather than blocking its interaction with CD55 (15). In this respect, it is highly interesting that in vitro internalization of 1B2 after binding to cellular CD97 was observed. Notably, this is the first report of internalization of an EGF-TM7 adhesion receptor, suggesting a novel mechanism of action for EGF-TM7 adhesion receptors and possibly other members of the adhesion-GPCR family. Until now internalization of adhesion-GPCRs has only been demonstrated for BA11, a member of subgroup VII of the adhesion-GPCR family, which can act as an engulfment receptor for apoptotic cells (32).

In conclusion, this study stresses the importance of careful examination of a target Ag, the various levels of its expression, and the fate of Ag-Ab complexes in the nondiseased and diseased situation when developing Ag-specific biotherapeutics. Furthermore, our data provide evidence that neutralization of CD97 is effective in experimental arthritis and may offer a novel approach for the treatment of patients with RA.

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Disclosures

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References


