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## Complement Activation Plays a Key Role in Antibody-Induced Infusion Toxicity in Monkeys and Rats **FREE**

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# Complement Activation Plays a Key Role in Antibody-Induced Infusion Toxicity in Monkeys and Rats

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Infusion reactions are a major side effect of the administration of therapeutic Abs and are the result of a complex immune reaction. In this study, we report that substitutions of Fc amino acids in the anti-HLA-DR Ab HD8 reduce its ability to induce infusion reactions in rats and monkeys. We first showed that i.v. administration of IgG1- and IgG2-subclass HD8 Abs induces severe infusion reactions in monkeys. These Abs express strong complement-dependent cytotoxicity (CDC), and in vivo depletion of complement in rats by pretreatment with cobra venom factor abrogated the lethal infusion reactions generated by HD8-IgG1. Thus, the infusion reactions appear to be largely driven by the complement system. To reduce the CDC function of HD8-IgG1, its Fc region was modified by two amino acid substitutions at Pro<sup>331</sup>Ser and Lys<sup>322</sup>Ala. The modified Ab was incapable of expressing CDC in vitro and did not induce severe infusion reactions in rats and monkeys, even at extremely high doses. The modified Ab retained its Ab-dependent cellular cytotoxicity function as well as its antitumor activity in a tumor-bearing mouse model. In summary, complement appears to drive infusion reactions, and modifications that eliminate the CDC activity of an Ab also reduce its ability to induce infusion reactions. *The Journal of Immunology*, 2008, 180: 2294–2298.

Infusion reactions are infusion-related side effects that occasionally occur, especially upon i.v. treatment with Ab-based drugs (e.g., rituximab and trastuzumab) (1–3). Infusion reactions manifest themselves as flu-like or anaphylaxis-like symptoms such as chills, asthenia, nausea, headache, rash, and vomiting. Several immune mechanisms have been suggested to participate in the induction of infusion reactions. First, the intensity of infusion reactions has been positively correlated with the rapid release of large amounts of cytokines, especially inflammatory cytokines. For example, the infusion of the anti-CD3 Ab OKT3 induces massive cytokine release (4, 5). Second, several therapeutic Abs have been suggested to activate the complement system, resulting in the production of large amounts of C3a, C4a, and C5a which can act as anaphylatoxins and induce the release of chemical mediators such as histamine and leukotriene (2, 6). Third, rituximab has also been suggested to induce the acute lysis of a large number of tumor cells via Ab-dependent cellular cytotoxicity (ADCC)<sup>2</sup> and/or complement-dependent cytotoxicity (CDC); this in turn induces an inflammatory reaction, i.e., infusion reaction, known as tumor lysis syndrome (7–9). The infusion reactions can be ameliorated to some extent by administering appropriate drugs and decreasing the infusion rate (10). Nevertheless, the precise mechanisms that lead

to infusion reactions and what can be done to prevent these reactions remain to be elucidated.

HLA-DR has been suggested to be a candidate target for Ab-based therapies against hematological malignancies. It has been shown that while the IgG1-type anti-HLA-DR Ab Hu1D10 induces infusion reactions (11–13), the IgG4-type anti-HLA-DR Ab 1D09C3 does not (14). Because IgG4 Abs do not evoke either ADCC or CDC, these observations suggest that the infusion reactions occurred by anti-HLA-DR Abs like Hu1D10 are induced by effector functions such as ADCC and/or CDC. In this study, we show that our anti-HLA-DR Ab HD8 (15) also induces an infusion reaction, and that this reaction correlates positively with the ability of this Ab to induce CDC. We also show that modifications of critical areas of the Fc region of HD8 that decrease the CDC potential of this Ab also reduce the risk of infusion reactions while retaining the ability of this Ab to induce ADCC against tumor cells.

## Materials and Methods

### Preparation of Abs

To obtain hybridomas producing anti-HLA-DR Abs, KM mice were immunized with HLA-DR-transfected L929 cells (CCL-1; American Type Culture Collection (ATCC)). KM mice were generated by the transchromosomal technique and produce fully human Abs upon immunization (16). The spleen cells from the immunized mice were then fused with the mouse myeloma cell line SP2/O-Ag14 (CRL-1581; ATCC) and the hybridoma producing HD8 was selected because it recognizes a wide variety of HLA-DR molecules (15).

To prepare the three rHD8-based Abs used in this study, the H and L chains expressed by the HD8 hybridoma were cloned into pGEM-T Easy Vector (Promega) and transferred into the N5KG1-Val Lark vector (Biogen IDEC). This generated the IgG1-type HD8 (HD8-IgG1) Ab. To make modified IgG1-type HD8 Abs that show reduced complement activation potential, standard point-mutation procedures were used to introduce the Pro<sup>331</sup>Ser alone and Pro<sup>331</sup>Ser plus Lys<sup>322</sup>Ala substitutions into the IgG1 Fc region of the HD8-IgG1 Ab (the EU numbering system (17) was used). The resulting Abs were designated as HD8-IgG1-Ser<sup>331</sup> and HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup>, respectively. The Abs were expressed in Chinese hamster ovary cells or HEK293 cells and purified from the culture supernatants by

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<sup>2</sup> Abbreviations used in this paper: ADCC, Ab-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; CVF, cobra venom factor.

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Table I. Ability of anti-HLA-DR Abs to induce infusion reactions in cynomolgus monkeys

Treatment	Dose (mg/kg)	Infusion Duration (min)	n	Infusion Reaction Score <sup>a</sup>
Vehicle	—	30	2	—
HD8-IgG1	0.003	30	1 <sup>b</sup>	—
	0.03	30		—
	0.3	30		—
	1.5	15		+++
HD8-IgG2	0.003	30	1 <sup>b</sup>	—
	0.03	30		—
	0.3	30		—
	1.5	15		+++

<sup>a</sup> The intensity of the infusion reaction was scored on the basis of monkey behavior described in *Materials and Methods*.

<sup>b</sup> The dose of each animal was escalated at hourly intervals.

using protein A (Amersham Biosciences). Flow cytometric analysis revealed that these HD8 Abs reacted to HLA-DR molecules with the same binding characteristics as the original Ab (data not shown). To generate Fab from the HD8 Ab, HD8-IgG1 was digested by immobilized pappain by using a Fab preparation kit (Pierce/Thermo Scientific).

### Animals

All rodents were maintained in air-conditioned, specific pathogen-free animal rooms with sterilized commercial rodent chow and water ad libitum. Cynomolgus monkeys were maintained in stainless steel cages in an air-conditioned room with commercial chow and water ad libitum. All experiments in this study were approved by the institutional animal care and use committee of the test facilities.

### In vitro cytotoxicity assays

For the ADCC assay, PBMCs were prepared from healthy volunteers by density centrifugation. For the CDC assay, human sera (Sigma-Aldrich) were used. Target Raji tumor cells (CCL-86; ATCC) were labeled with <sup>51</sup>Cr-sodium chromate (PerkinElmer) for 1 h at 37°C. For the ADCC assay, the target cells were mixed with the PBMCs and Abs, and incubated at 37°C for 4 h. For the CDC assay, the target cells were mixed with human sera and Abs, and incubated at 37°C for 2 h. Tumor cell lysis was measured by determining the amount of <sup>51</sup>Cr released into the supernatant by a scintillation counter. The percentage of specific lysis was determined by the following equation:  $100 \times (\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})$ . To perform the ADCC and CDC assays using rat effectors, the procedures described above were followed using PBMCs or spleen cells (ADCC assay) and sera (CDC assay)

from WKAH rats (Japan SLC). All human specimens used were approved by the institutional ethical committee of our facility.

### Nonhuman primate study

Ten 3- to 7-year-old male cynomolgus monkeys were used in total. We first examined whether HD8 could recognize their CD20-positive PBMCs known to express MHC class II Ags by flow cytometric analyses. All monkeys were strongly recognized by HD8 (data not shown). To evaluate the toxic effects of HD8 and its Fc variants, the monkeys were given the Abs or vehicle (PBS containing 1% monkey serum) i.v. by infusion pumps (BSP-99M; Braintree Scientific) and their behavior was monitored. Typical symptoms of infusion reaction are passive behavior, recumbency, pallor, nausea, vomiting, and in more severe cases, hypothermia, hyperventilation, and occasional death, which are expressed within a few hours. The intensity of these infusion reactions was scored as follows: + + +, strong reaction (e.g., severe hypothermia and hyperventilation with occasional mortality); + +, intermediate reaction (e.g., mild hypothermia and long-term recumbency without mortality); +, weak reaction (e.g., passive behavior and short-term recumbency); and —, no abnormal change.

### Rat infusion reaction model

Various rats were screened by flow cytometry to identify strains which are recognized by HD8. The WKAH rat (Japan SLC) was selected on the basis of the high reactivity of its PBMCs with HD8 (data not shown). Five-week-old male WKAH rats were bolus-injected with various Abs i.v. and their behavior was monitored for 5 h continuously and daily thereafter. The infusion reaction symptoms were scored as described above. Seriously injured animals were euthanized. To determine whether the infusion reaction correlates with serum complement, the WKAH rats were injected i.v. with cobra venom factor (CVF) (18) from *Naja melanoleuca* (US Biological) at 100 µg/animal (~1 mg/kg) 5 h before the administration of the HD8 Abs.

### In vivo antitumor activity on mice

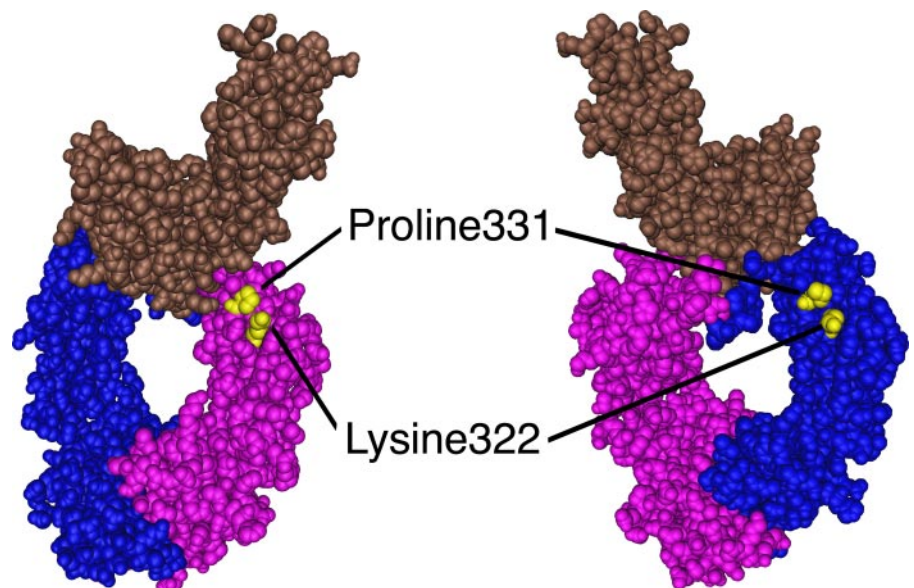
To generate a lymphoma-xenografted model, 6- to 8-wk-old *scid* mice were injected with 0.01 ml of anti-asialo GM1 antisera (Wako Pure Chemical) 1 day before (day -1) the i.v. inoculation of  $5 \times 10^6$  Raji cells (day 0). On day 5, the mice were injected i.v. with 500 µg/kg HD8-IgG1, HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> or control Ab and their survival was monitored daily. The survival data were analyzed statistically by the log-rank test.

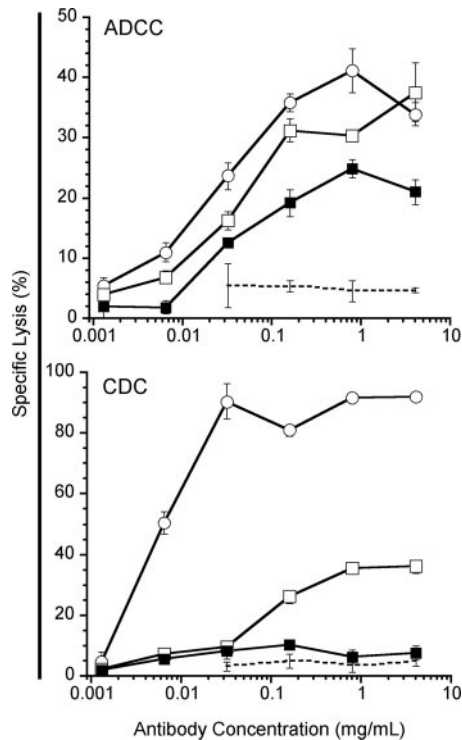
## Results

### Toxicity of anti-HLA-DR Abs in cynomolgus monkeys

The Ab HD8, which we previously reported to recognize a wide range of HLA-DR molecules (15), was examined for its ability to induce infusion reactions in cynomolgus monkeys. All monkeys used in this study were confirmed by flow cytometric analysis to react strongly with the HD8 Ab (data not shown). Two monkeys

**FIGURE 1.** Positions of the substituted Fc amino acids in the Fc/FcR complex. The locations in the tertiary structure of human IgG1 Fc of the amino acids that were substituted in the modified HD8 Abs are shown. Two CH2-CH3 domains of the IgG1 Fc region and the FcγRIII (cited from Protein Data Bank, No. 1E4K) are shown in purple/blue and brown, respectively. The lysine 322 and proline 331 residues are highlighted in yellow.





**FIGURE 2.** Ability of the Fc-variant HD8-IgG1 Abs to induce ADCC and CDC in vitro. The HD8-IgG1 (○), HD8-IgG1-Ser<sup>331</sup> (□), HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> (■), and control Abs (dotted line) were incubated at various doses with <sup>51</sup>Cr-labeled Raji target cells along with (top) healthy human PBMCs as effector cells at an E:T ratio of 100 for 4 h (ADCC assay), or with (bottom) 5% human sera for 2 h (CDC assay). The specific lysis ratios were then calculated by the formula described in *Materials and Methods*. The bars represent the SEMs of triplicates. Repeated experiments yielded similar and consistent results.

were infused with the vehicle by using infusion pumps while one monkey received the IgG1-type Ab HD8-IgG1 and another received the IgG2-type Ab HD8-IgG2. The Abs were administered initially at 0.003 mg/kg. At hourly intervals, the dose was escalated to 0.03, 0.3, and 1.5 mg/kg. The behavior of the animals was monitored and scored to provide a measure of the intensity of the infusion reaction (Table I). Neither Ab induced abnormal changes at doses of 0.003, 0.03, and 0.3 mg/kg, but severe reactions were observed at the 1.5 mg/kg dosing of both Abs. Indeed, both mon-

keys died after this dose was delivered. The two control monkeys did not manifest any infusion reaction symptoms.

#### *In vitro cytotoxicity assays by human effectors*

Because the infusion reactions of anti-HLA-DR Abs were shown to be induced by IgG1-type (12, 13) and IgG2-type Abs (Table I), both of which possess strong CDC, and not by IgG4 Ab with no CDC (14), we tested whether the infusion reactions generated by Ab therapies arise from CDC. We generated rHD8-IgG1 Abs bearing the Pro<sup>331</sup>Ser alone and Pro<sup>331</sup>Ser plus Lys<sup>322</sup>Ala substitutions in their Fc regions. These substitutions were speculated to reduce the CDC potential of HD8-IgG1 without affecting its ADCC function (19, 20). The locations of amino acids 322 and 331 in the Fc region of IgG1 are indicated in Fig. 1, which shows the tertiary structure of the IgG1 Fc bound to the FcR (cited from Protein Data Bank Database No. 1E4K) (21, 22). Residues 322 and 331 do not overlap directly with the binding site of the FcR. To test the effect of the substitutions on HD8-IgG1 cytotoxicity activity, HD8-IgG1 and these variants were then subjected to in vitro ADCC and CDC assays using human PBMCs and sera, respectively. While HD8-IgG1 evoked both ADCC and CDC, HD8-IgG1-Ser<sup>331</sup> generated markedly weaker CDC and slightly weaker ADCC, while HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> induced weaker ADCC and no CDC at all (Fig. 2). Thus, while the Abs bearing one or both of the two amino acid substitutions still evoked some ADCC, their ability to induce CDC was decreased or absent in comparison with the original IgG1 Ab.

#### *Ability of HD8 Fc variant Abs to induce infusion reactions in a rat model*

To test the effect of the Fc residue substitutions on the ability of HD8-IgG1 to induce infusion reactions, we established a rat infusion reaction model. Various rodent strains were screened to identify strains recognized by HD8-IgG1. The rat strain WKAH was recognized strongly by HD8 (data not shown) and thus this strain served as the infusion reaction model.

Initially, we examined the ability of HD8-IgG1 and its Fc variants to induce ADCC and CDC in vitro by using WKAH rat PBMCs and sera, respectively (Table II). HD8-IgG1, HD8-IgG1-Ser<sup>331</sup>, and HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> all induced ADCC equally well but HD8-IgG1-Ser<sup>331</sup> evoked CDC more weakly than HD8-IgG1, while HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> did not induce CDC at all. We then examined the abilities of these Abs to evoke an infusion reaction in the rat model. Thus, the rats were infused i.v. with 1, 3, or 10 mg/ml Ab by bolus administration, and their behavior was monitored and scored to indicate the intensity of the infusion

Table II. Ability of HD8 Abs to induce ADCC and CDC in vitro and infusion reactions in vivo in the WKAH rat model

Group	Treatment	Ab		Dose (mg/kg)	Score <sup>a</sup>	
		Name	ADCC <sup>b</sup>			CDC <sup>b</sup>
1	None	HD8-IgG1	+++	+++	1	++
			3	+++		+++
2	None	HD8-IgG1-Ser <sup>331</sup>	+++	+	3	+
			10	+++		++
3	None	HD8-IgG1-Ala <sup>322</sup> Ser <sup>331</sup>	+++	-	3	+
			10	+++		+
4	None	HD8-Fab	-	-	10	-
5	None	Control IgG1	-	-	10	-
6 <sup>c</sup>	CVF	HD8-IgG1	+++	+++	3	+

<sup>a</sup> The intensity of the infusion reaction was scored on the basis of rat behavior described in *Materials and Methods*.

<sup>b</sup> The ability of the Abs to evoke ADCC and CDC in vitro when WKAH rat effectors (PBMCs and sera, respectively) were used was scored as +++, ++, +, and -.

<sup>c</sup> Group 6 animals were given CVF (100 μg/rat) 5 h before a lethal dose of HD8-IgG1 (3 mg/kg) was administered.

Table III. Ability of HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> to induce infusion reactions in cynomolgus monkeys

Ab	Dose (mg/kg)	Infusion Duration (min)	n	Score <sup>a</sup>
Vehicle	—	240	3	—
HD8-IgG1-Ala <sup>322</sup> Ser <sup>331</sup>	50	240	3	—

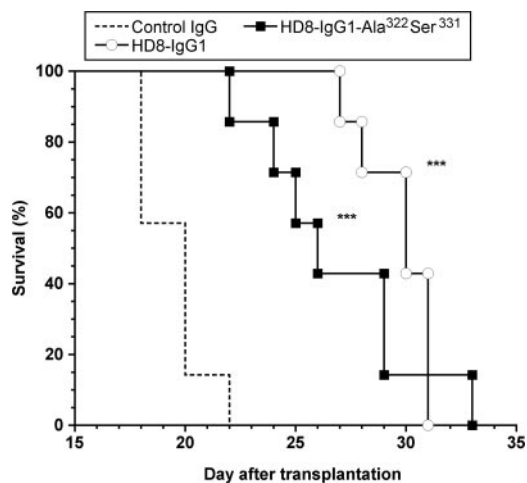
<sup>a</sup> The intensity of the infusion reaction was scored on the basis of monkey behavior described in *Materials and Methods*.

reaction (Table II). Neither the Fab of HD8-IgG1 nor the control IgG generated abnormal signs, even at 10 mg/kg. However, HD8-IgG1 induced severe infusion reactions: one of the three rats receiving 1 mg/kg died while all three receiving 3 mg/kg died. In contrast, HD8-IgG1-Ser<sup>331</sup> induced a weak infusion reaction at 3 mg/kg and lethal toxicity was not observed, although at 10 mg/kg, it killed three of five rats. HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> only evoked a weak infusion reaction at both 3 and 10 mg/kg and lethal toxicity was not observed. Thus, the severity of the infusion reactions followed the order HD8-IgG1 > HD8-IgG1-Ser<sup>331</sup> > HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> >> Fab and control IgG. This correlates positively with the ability of the Abs to evoke CDC. Thus, decreasing the ability of the HD8 Ab to evoke CDC also reduces its ability to induce infusion reaction in rats.

To test this notion further, we pretreated WKAH rats with C566, which is known to activate and deplete complement (18). Thus, four WKAH rats were injected i.v. with C566 5 h before a lethal dose of HD8-IgG1 (3 mg/kg) was administered (Table II). The administration of C566 itself did not induce any abnormal changes. HD8-IgG1 induced an infusion reaction in the C566-treated rats briefly but all rats returned to normal within 2 h. These results strongly suggest that complement activation is one of the major causes of the infusion reactions induced by the anti-HLA-DR Ab HD8.

#### Ability of HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> to induce infusion reactions in cynomolgus monkeys

We then examined whether HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> induces infusion toxicity in cynomolgus monkeys (Table III). Three monkeys



**FIGURE 3.** In vivo antitumor activity of HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup>. HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> prolongs the survival of lymphoma-xenografted mice. *scid* mice were pretreated with anti-asialo GM1 antisera on day -1, inoculated with Raji lymphoma cells i.v. on day 0, and then treated with 50  $\mu$ g/kg HD8-IgG1 (○), HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> (■), or control IgG (dotted line) on day 5. \*\*\*, Significant differences in comparison with control IgG-administered mice, as determined by the log-rank test (\*\*\*,  $p < 0.001$ ).

were given vehicle or HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> at a dose of 50 mg/kg i.v. by 4-h infusion (12.5 mg/kg/h). Remarkably, infusion reactions were not observed in any of the monkeys.

#### In vivo antitumor activity of HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> in a mouse xenograft model

*scid* mice were xenografted i.v. with Raji cells, treated i.v. with 500  $\mu$ g/kg HD8-IgG1 or HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> 5 days later, and monitored for survival (Fig. 3). All mice given the control IgG died within 22 days of transplantation. In contrast, HD8-IgG1 and HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> significantly prolonged the lifespans of the mice (both  $p < 0.001$ ). Although the half-lives of the HD8-IgG1- and HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup>-treated mice were 26 and 30 days, respectively, this difference was not statistically significant.

## Discussion

Therapeutic Abs sometimes induce infusion reactions, and this can occasionally be life-threatening. Currently, such reactions are reduced or prevented by alterations in clinical practice, for example, by slowing the infusion rate, instituting prophylactic treatment with antihistamine drugs, and treating the symptoms with corticosteroids (1). We show here directly for the first time that severe infusion reactions can be avoided by modifying the Fc region of the therapeutic Ab.

Consistent with what has been observed previously (1, 2, 6), we showed that the severity of the infusion reactions generated by the Abs is associated with the activation of the complement system because our prototype IgG1- and IgG2-subclass anti-HLA-DR Abs did not just induce severe infusion reactions in both rats and monkeys, they also evoked strong CDC responses when human and rat sera were used as the effector source. This association between infusion reactions and the complement system was confirmed when we pretreated rats with C566, which depletes complement, before administering a lethal dose of HD8-IgG1: these rats suffered only a mild infusion reaction.

To reduce this CDC function of the HD8 Ab, we sought to modify its Fc region. We could have chosen to express the Ab as a IgG4-subclass Ab, as such Abs lack both CDC and ADCC functions. Indeed, Nagy et al. (14) have shown that an anti-HLA-DR Ab of this subclass does not induce infusion toxicity in monkeys. However, rituximab has been shown to be effective against non-Hodgkin's lymphoma because it elicits ADCC (23, 24). Consequently, making HD8 as IgG4 Ab is likely to eliminate its antitumor ADCC activity as well. We were then faced with the problem that modifications of the Fc region that eliminate CDC may also affect ADCC because the C1q-binding site overlaps with the FcR-binding site (19–21, 25–27). However, when we substituted residues 322 and 331 in the HD8 Fc region—these residues do not overlap exactly with the FcR-binding site (Fig. 1)—the CDC function of the Ab was lost while both its ADCC and in vivo antitumor functions were retained. Moreover, the modified Ab did not induce infusion reactions in cynomolgus monkeys at the massive dose of 50 mg/kg, which exceeds the lethal dose of HD8-IgG1 (1.5 mg/kg) by 30-fold. These results suggest that similar modifications may be useful for other Abs whose therapeutic use is limited by their propensity to induce infusion reactions. Moreover, these results further support the notion that infusion reactions are largely caused by CDC.

Klingbeil and Hsu (12) have shown previously that the humanized IgG1 anti-HLA-DR Ab Hu1D10 induces severe infusion reactions in monkeys. Moreover, in clinical trials with this Ab, some patients suffered infusion reactions and had to be given antiana-phylactic medications (13). Such concerns are serious impediments to the clinical use of HLA-DR-targeting drugs for treating B

cell malignancies, including non-Hodgkin's lymphoma. Thus, our modified HD8-IgG1-Ala<sup>322</sup>Ser<sup>331</sup> anti-HLA-DR Ab may be highly useful in the clinic.

To test the effect of the Fc modifications on the ability of HD8 to induce CDC and ADCC and control tumor growth in vivo, we established a novel infusion reaction rat model. The WKAH rat strain that was used was selected because preliminary studies revealed its PBMCs were strongly recognized by HD8; moreover, there was high homology between the HD8 epitope (15) and the MHC class II sequences of the rats. That HD8 acts similarly in both this rat model and the cynomolgus monkey model was revealed by the fact that it had similar ADCC and CDC bioactivities in vitro and induced similar infusion reaction symptoms in both species. The rat model did differ from the monkey model in that the Abs were administered by bolus injection rather than by slow infusion; the former method permits the administration dose for the induction of infusion reaction to be lowered.

Despite the strong correlation between infusion reaction severity and the CDC activity of the HD8 Ab, it is likely that other immune mechanisms also contribute to infusion reactions because our modified CDC-incompetent Ab still induced some mild infusion reactions in the rat model. One possible additional mechanism is that the binding of HD8 Abs to HLA-DR on mast cells activates them; indeed, it has been shown that such binding induces ultrastructural mast cell changes that suggest they are induced to secrete granules (28). However, this possibility is less likely because the Fab of the HD8 Ab did not induce infusion reactions in the rat model. Moreover, an IgG4-type anti-HLA-DR Ab does not induce infusion toxicity on monkeys (14). Thus, it is unlikely that the direct binding of the Ab to HLA-DR plays a significant role in inducing infusion reactions. The additional mechanisms contributing to infusion reactions remain to be elucidated.

In conclusion, we demonstrated that 1) the severity of infusion reactions induced by an anti-HLA-DR Ab correlate strongly and positively with its ability to evoke CDC, and that 2) substitutions of two amino acids on the Fc region that diminish CDC also reduce the risk of infusion reaction without diminishing the antitumor activity of the Ab.

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## Disclosures

All authors are employed by Kirin Pharma whose potential product was studied in the present work. T. Tawara and S. Kataoka hold a patent related to the work that is described in the present study.

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