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A role of the Duffy antigen for the maintenance of plasma chemokine concentrations

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Abstract

We examined plasma chemokine concentrations and chemokine clearance rates in Duffy antigen knockout mice. The plasma concentrations of eotaxin and MCP-1 in Duffy antigen knockout mice were less than one-third of those in wild-type mice. When eotaxin or hMGSA was intravenously injected, the chemokine disappeared more rapidly from the plasma of Duffy antigen knockout mice than from the plasma of wild-type mice. The half-lives of hIP-10 and interferon-γ, which do not have an affinity for the Duffy antigen, in plasma were indistinguishable between Duffy antigen knockout mice and wild-type mice. These results suggest that the Duffy antigen delays the disappearance of chemokines from the plasma, resulting in the maintenance of plasma chemokine concentrations.

Chemokines are protein factors that attract immune cells and are involved in numerous aspects of immunologic processes, including leukocyte trafficking, inflammation, angiogenesis, tumor growth, and suppression of human immunodeficiency virus (HIV) [1]. Chemokines are classified as either CC chemokines or CXC chemokines according to their structures. CC chemokines attract mononuclear cells, whereas CXC chemokines attract mainly granulocytes. Clinical trials for the therapeutic use of chemokines were recently reported [2]. For appropriate therapeutic use of protein factors, an understanding of the environmental and genetic factors that affect their pharmacokinetics is essential [3]. Studies of chemokine pharmacokinetics in animals and humans have been reported [4–6]. Little is known, however, regarding the genetic factors affecting chemokine pharmacokinetics.

Erythrocytes have high affinity for both CC and CXC chemokines [7–9]. Once chemokines bind to erythrocytes, they lose their ability to attract target cells. The Duffy antigen on erythrocyte membranes is responsible for interactions with chemokines [10–12]. Previous reports claimed that the Duffy antigen suppresses an increase in plasma chemokine concentrations due to absorption of the chemokines [7]. Dawson et al. [13] reported that the exaggerated inflammatory response is observed in Duffy antigen knockout mice and mentioned that this phenotype is consistent with the idea that Duffy antigen functions as a chemokine sink. However, Luo et al. [14] reported contradictory observation to this notion.

In the present study, we compared plasma chemokine concentrations and chemokine clearance rates between Duffy antigen knockout mice and wild-type mice. Our results demonstrate that the Duffy antigen functions to maintain plasma chemokine concentrations.

Materials and methods

Generation of Duffy antigen knockout mice. We constructed a targeting vector that replaced the exon1 containing the initial codon and a 5′ site of exon2 of the Duffy antigen gene to the neomycin resistant gene (data not shown). We isolated the 15-kb genomic DNA fragment containing the Duffy antigen gene from a mouse genomic library using

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the Duffy antigen cDNA as a probe. The 4.5-kb *Xba*I genomic DNA fragment (5′ arm) located in the 5′ upstream region of exon1 of Duffy antigen gene and the 8.2-kb *Xho*I–*Cla*I genomic DNA fragment (3′ arm) containing the 3′ site of exon2 were cloned to the plasmid pMH003 harboring the neomycin resistant gene and the Diphtheria toxin A gene. The linearized targeting vector (50 μg) by *Not*I was transfected by electroporation into 3×10^7 E14 cells (provided by Prof. Katsuki, National Institute for Basic Biology) and neomycin resistant clones were isolated. The homologous recombinant clones were determined by Southern blot hybridizaton. The homologous recombinants were injected into mouse blastocysts (C57BL/6J) and chimeric mice were obtained. The resulting male chimeras were mated with female C57BL/6 mice, followed by selecting germline transmitters. The genotypes of the offspring were determined using Southern blot analysis with the 5′ probe (data not shown). All mice were maintained in a specific pathogen-free animal facility at the University of Tokyo.

Endogenous chemokine plasma levels. Blood was harvested from the tail vein of 9- to 12-week-old mice. Plasma was prepared by centrifugation of the blood at 3000*g* for 20 min (Kubota 3100). Cytokines were determined by a sandwich ELISA. ELISA kits and chemokines were purchased from R&D Systems (Minneapolis, MN).

Table 1
Plasma concentrations of chemokines in mice

Chemokine	Genotype of the Duffy gene	
	+/+	−/−
Eotaxin	$4200 \pm 1800^*$	$930 \pm 30^*$
MCP-1	110 ± 40	<40

Blood samples were collected from tails using heparinized capillary tubes and plasma concentrations (pg/ml) of chemokines were determined by ELISA.

$^*P < 0.05$, means ± SD.

Result and discussion

Lower plasma chemokine concentrations in Duffy knockout mice

We compared plasma concentrations of eotaxin and MCP-1, chemokines that have a binding affinity for the Duffy antigen, between wild-type and Duffy antigen knockout mice. Blood samples were taken from tails and chemokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA). The results indicated that the plasma concentration of eotaxin in Duffy antigen knockout mice was one-fifth that of wild-type mice (Table 1). The concentration of MCP-1 was also significantly lower in Duffy antigen knockout mice than in the wild-type mice.

Rapid disappearance of chemokines from plasma in Duffy knockout mice

We hypothesized that the lower plasma concentrations of eotaxin and MCP-1 in Duffy antigen knockout mice were caused by rapid clearance of these chemokines. To test this, we compared the disappearance rates

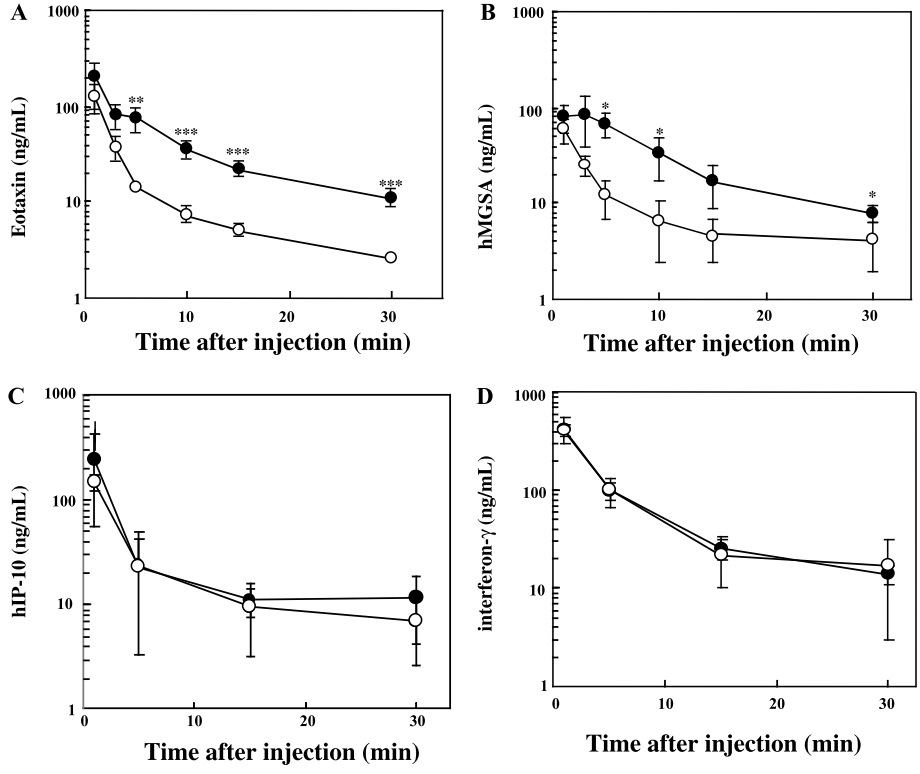

Fig. 1. Clearance of eotaxin, hMGSA, hIP-10, and interferon-γ from plasma. After intravenous injection of chemokines, blood samples were collected from tails using heparinized capillary tubes. Chemokines in plasma were determined by ELISA. Eotaxin (A), hMGSA (B), hIP-10 (C), and interferon-γ (D). Wild-type mice (closed circle), knockout mice (open circle). $^{***}P < 0.005$, $^{**}P < 0.02$, and $^*P < 0.05$, $n = 3$–5, means ± SD.

of intravenously injected chemokines from the plasma of Duffy antigen knockout and wild-type mice. The half-lives in plasma of eotaxin and hMGSA, a CC chemokine and a CXC chemokine, respectively, administered by single intravenous injection to Duffy antigen knockout mice were one-third those of wild-type mice (Figs. 1A and B). The half-lives in plasma of MCP-1, hIL-8, and MIP-2, administered by single intravenous injection to Duffy antigen knockout mice, were also significantly lower than those of wild-type mice (data not shown). In contrast, the half-lives of hIP-10 (Fig. 1C), hMIP-1β (data not shown), interferon-γ (Fig. 1D), and tumor necrosis factor-α (data not shown), which do not bind to the Duffy antigen, in plasma were indistinguishable. Therefore, the rapid disappearance from the plasma of Duffy antigen knockout mice might be a characteristic feature of chemokines that have an affinity for the Duffy antigen. When [^{125}I]hMGSA was administered to Duffy antigen knockout mice, the chemokine was immediately absorbed by the liver and kidney (data not shown), suggesting that chemokines are excreted from these organs.

Previous reports suggested that the Duffy antigen absorbs chemokines and stimulates the disappearance of chemokines from the plasma [7]. Our results obtained using gene knockout mice indicate that the Duffy antigen delays the disappearance of chemokines from the plasma. We propose that the Duffy antigen acts as a reservoir of chemokines and releases them when plasma chemokine concentrations decrease. The K_d values of chemokines for the Duffy antigen are reported to be less than 10 nM [8–12]. When plasma chemokine concentrations decrease below the K_d value, the chemokines that are bound to the Duffy antigen on erythrocyte membranes might be released, resulting in the maintenance of plasma chemokine concentrations. The "sink" hypothesis and the "reservoir" hypothesis might not be exclusive of each other. Duffy antigen might act as a sink of chemokines in the local microenvironment where inflammation occurs, whereas it could act as a reservoir of chemokines in the plasma.

A large number of humans have genetic defects of the Duffy antigen. For example, half of American blacks have no Duffy antigen on their erythrocytes. Plasma chemokine concentrations in these people might be maintained at a low level. Chemokines are suggested to be involved in HIV suppression [15]. It is possible that there are differences in the susceptibility to HIV between Duffy antigen-negatives and Duffy antigen-positives.

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