- Anti-human HRG polyclonal antibody(α-hHRG pAb)：岡山大学大学院・医歯薬総合研究科・薬理学
- Super Signal West Dura Extended Duration Substrate：Thermo Scientific
- 細胞培養用 10 cm dish(10 cm dish) ： BD FALCON
- 細胞培養用 35 mm dish(35 mm dish) ： BD FALCON

- ニトロセルロース膜：BIO RAD
- Image Quant Las 4000mini：GE healthcare
- 電気泳動槽（MODEL BE-222）：BIO CRAFT・細胞培養用 5 mL フラスコ(5 mL フラスコ) ： SIGMA

- 細胞培養用 6 ウェルプレート ： BD FALCON

2-5-2　試薬調整
・M-PER 液
無菌、常温の条件下で 540 μL の M-PER に 10 μL の PI カクテルを溶かした。

・選択培地
無菌、常温の条件下で 50 mL の DMEM/F12(1:1)(1x)に 50 μL の 10 mg/ mL ピューロマイシンを溶かした。

2-5-3　実験方法
　リポフェクション法により HEK293 細胞にベクターPCMVi-C-OriP-HRG を導入し 37 ℃ 5 %CO₂で 48 時間培養した。

　48 時間後、選択培地で HEK293 細胞を 37 ℃、5 %CO₂で 1 週間培養後、培地を交換し、以降 4 日間毎に培地交換した。

　形成されたコロニーは回収し、選択培地を用いて 1 枚の 6 ウェルプレート、2 枚の 35 mm dish で細胞が 80 %コンフルエントになるまで培養した。そして、培養上清を回収し rHRG の発現を Western Blotting 法で確認した。Western Blotting は氷中、100 V、90 分の条件で転写を行った。以降の操作は第一章 1-3-3 [実験方法]と同様に行った。また、Western Blotting により得られたバンドの発光強度の測定はバンドの輝度を測定するソフトウェア Image Quant TL を用いた。
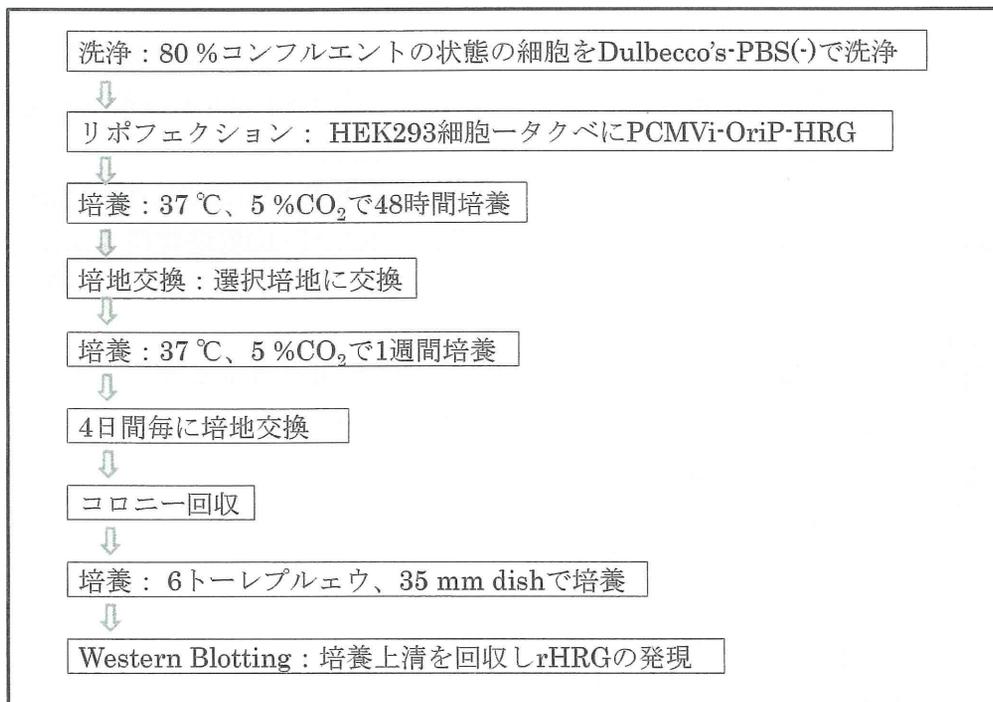
洗浄：80％コンフルエントの状態の細胞をDulbecco's-PBS(-)で洗浄

⇩

リポフェクション：HEK293細胞ータクベにPCMVi-OriP-HRG

⇩

培養：37℃、5％CO_2で48時間培養

⇩

培地交換：選択培地に交換

⇩

培養：37℃、5％CO_2で1週間培養

⇩

4日間毎に培地交換

⇩

コロニー回収

⇩

培養：6トーレプルェウ、35 mm dishで培養

⇩

Western Blotting：培養上清を回収しrHRGの発現

表8：HEK293 細胞と rHRG 発現ベクターPCMVi-C-OriP-HRG を用いた rHRG 安定発現株の作製プロトコール

2-5-4 結果

図 2-8 から、HEK293 細胞で安定発現株化細胞の作製を試みたが、作製できなかった。
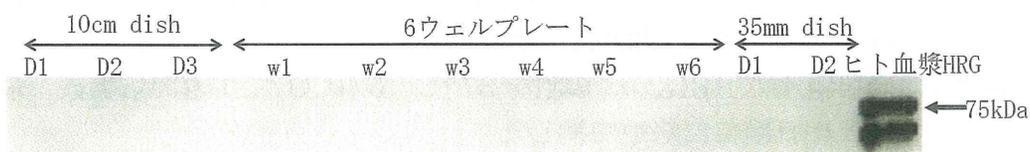

図 2-5：HEK293 細胞と rHRG 発現ベクターCMVi-C-OriP-HRG を用いた
rHRG 安定発現株の作製結果

溶出画分は約 13.3 μl、ヒト血漿 HRG 精製標品(25 ng)

10 cm dish D1、2、3：3 枚の 10 cm dish で PCMVi-C-OriP-HRG 導入 HEK293 細胞を培養した上清
6 ウェルプレート w1、w2、w3、w4、w5、w6：各ウェルに PCMVi-C-OriP-HRG 導入 HEK293 細胞を培養した上清
35 mm dish 培養上清 D1、2：2 枚の 35 mm dish で PCMVi-C-OriP-HRG 導入 HEK293 細胞を培養した上清

2-5-5 考察

　哺乳動物由来細胞を用いた rHRG 作製の意義は大腸菌系では行われない糖鎖付加が行われる点にあり、ヒト血漿 HRG と同様の活性を示す HRG の産生には重要であると考えられた。そして、HRG を医薬品として開発する場合、rHRG を安定的に大量産生する系の確立は必須となり、4 種類の HRG 発現ベクターを使用して rHRG の発現量を確認し、rHRG 安定発現株の作製を試みた。

　HRG 発現ベクターPCMVi-C-TSC-HRG を HEK293 細胞、HepG2 細胞に導入した結果、HEK293 細胞、HepG2 細胞が産生した全 rHRG 量はそれぞれ約1655.5 µg、350.2 µg であり、各細胞における rHRG 分泌率は HEK293 細胞では約 96.8 %、HepG2 細胞では約 98.5 %と HepG2 細胞の方が 1.7 %分泌効率が高かった(図 2-2)。

　リポフェクション法による目的タンパク質の強制発現機序は核内に移行したベクターのプロモーターが核内の転写因子と結合する事で目的タンパク質の発現が開始される。したがって、目的タンパク質の発現量の決め手はベクターと転写因子との結合性が重要であると考えた。

　rHRG 発現ベクターPCMVi-R-TSC-HRG を用いた場合、rHRG の産生能力は HEK293 細胞の方が HepG2 細胞と比較して約 4.7 倍多いが、rHRG への糖鎖修飾は rHRG 分泌効率から HepG2 細胞が優れていると考えた。

　HepG2 細胞の転写因子よりも HEK293 細胞の転写因子と HRG 発現ベクター中のプロモーター配列の相性が良かったため rHRG 産生量は HEK293 細胞が多くなったと考えた。

　また、細胞間の糖鎖修飾に関して HRG は肝臓由来の糖タンパク質である事からも HEK293 細胞よりも HepG2 細胞の方が rHRG への糖鎖修飾に最適な条件が整っていると考えられた。

　本研究では rHRG の安定的な大量産生系を確立するため、ベクターPCMVi-R-TSC-HRG で rHRG の発現量が多い HEK293 細胞を用いる事とした。

　4 種類のベクター(PRET-CMVi-R-TSC-HRG、PCMVi-C-OriP-HRG、PRET-CMVi-C-HRG、CMVi-R-TSC-HRG)を HEK293 細胞に導入した結果、ベクターPCMVi-C-OriP-HRG、PRET-CMVi-C-HRG、CMVi-R-TSC-HRG で rHRG の発現が確認できたが、ベクターPRET-CMVi-R-TSC-HRG では rHRG の発現は確認できなかった(図 2-5)。rHRG の発現量は PCMVi-C-OriP-HRG で最大になる事が分かった(図 2-5)。

　ここで、4 種類のベクター間における rHRG 発現量の違いはベクターの構造に起因すると考えた。4 種類のベクター全てに CMV プロモーターが組み込まれているが、PRET-CMVi-R-TSC-HRG には LTR 配列及び RU5'配列が組み込まれ、PCMVi-C-OriP-HRG には LTR 配列及び RU5'配列が組み込まれていない。

また、PRET-CMVi-R-TSC-HRG には LTR 配列のみが組み込まれ、CMVi-R-TSC-HRG には RU5'配列のみが組み込まれていた。

　LTR 配列はレトロウイルスゲノムの両端にある反復配列で、転写制御を行うプロモーターやエンハンサー等導入した HRG 遺伝子を細胞ゲノムに挿入するために組み込まれている。また、RU5'配列は HIV 由来の塩基配列で、HRG 遺伝子の転写を促進するために組み込まれている。

　LTR 配列及び RU5'配列は HEK293 細胞と相性が悪く、HEK293 細胞の転写因子と最も相性の良いプロモーターは CMV プロモーターのみであると考えられた(図 2-5)。

　また、細胞を洗浄した際に得られた PBS(-)から rHRG が検出された(図 2-5)。HRG は細胞表面に発現しているヘパラン硫酸と結合する事が知られており、HEK293 細胞が産生した rHRG はヒト血漿 HRG のヘパラン硫酸結合性を持つ事が示唆された(図 2-5)。

　HEK293 細胞にベクターPCMVi-C-OriP-HRG を導入し、HRG 安定発現株作製を試みた結果、 rHRG 安定発現株を作製する事はできなかった(図 2-6)。

　原因としてベクターが細胞の染色体に組み込まれる過程に問題があったと考えられた。ベクターが細胞の核内に移行した場合、ベクターが分断される。この過程で HRG 遺伝子を含まない塩基配列、HRG 遺伝子を分断した塩基配列、HRG 遺伝子を含むがピューロマイシン遺伝子を含まない塩基配列、ピューロマイシン耐性遺伝子のみの塩基配列を持った細胞のコロニーが形成されたと考えられた。

結語

　高ヒスチジン糖タンパク質(HRG)は多様な分子と結合性を示す事を冒頭で紹介した。ヒト血漿から HRG を精製する場合、HRG 結合性分子を利用した精製法が考えられるが、この場合 HRG 結合性分子の担体作製という操作を経なければならず簡便ではない。

　そこで、本研究では Ni-NTA 担体と HRG 分子内のヒスチジンとの結合性を利用して HRG 精製を行った。その結果、Ni-NTA 担体によりヒト血漿から HRG の粗精製に成功した。

　さらに、夾雑タンパク質除去の目的で陰イオン交換クロマトグラフィーを行った。その結果、ほとんどの夾雑タンパク質が除去され HRG 精製標品が得られた。Ni-NTA 担体を用いたヒト血漿からの HRG 精製は簡便であると考えられた。

　ここで、HRG を医薬品として開発する観点から、ヒト血漿を利用した場合安全性や安定供給の面が問題になると考えられ、この問題の解消には HRG を安定に産生する系の確立が必須であると考えられた。

そこで、4種類のHRG発現ベクターをHEK293細胞に導入し最もrHRGを発現するベクターでHRG安定発現株の作製を行ったが、細胞ゲノムへのHRG遺伝子及び薬剤耐性遺伝子の導入に問題が生じHRG安定発現株の作製は出来なかった。

　しかし、HRG安定発現株の作製は先に述べた医薬品としてのHRG開発には必須の上rHRGの機能解析に供する標品取得の観点からも今後とも継続してHRG安定発現株の作製に取り組んでいく方針である。

CHO 細胞を用いた rHRG 一過性発現株、安定発現株の作製

　平成２４年１２月３日に PMDA 面談を実施していただき、特にバイオ製剤開発において留意すべき点についてアドバイスを受けた。その中で原薬選定にいたる過程については、十分検討するように示唆された。その際一つの考え方として、既存のバイオ製剤作製でもっとも頻用されている哺乳類細胞 Chinese hamster ovary cell（CHO）を用いることは、基礎的な作製条件のチェックに関し、省略できる部分が多いことが示唆された。原薬選定への道筋を描き易いことは大きなメリットである。したがって、本研究の中でも、先行する HEK293 細胞を用いたヒト組換え HRG 作製と並んで CHO 細胞を用いた発現系を確立する。
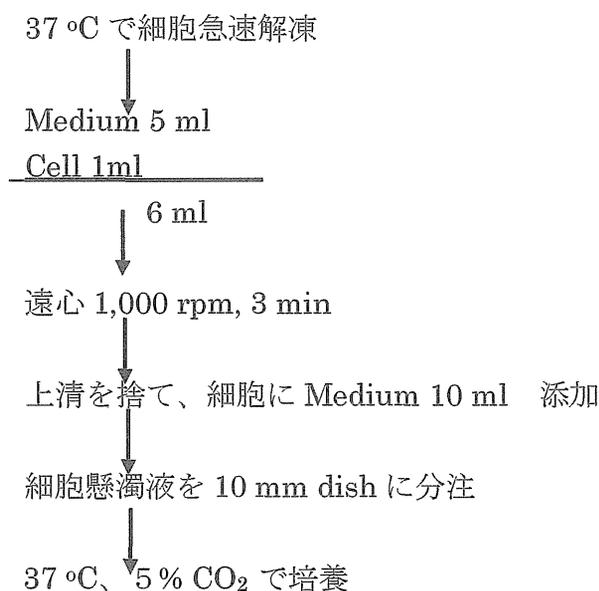
第一節　CHO-k1 と CHO-k1-sc 細胞の培養

「プロトコール１」
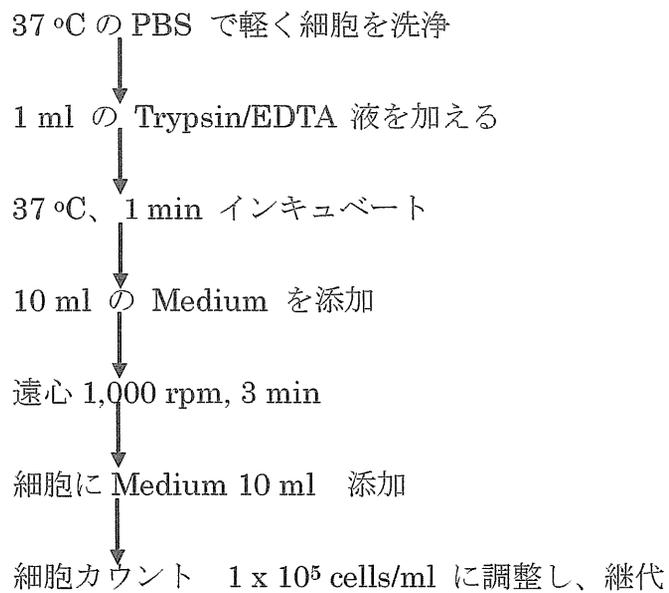CHO-ｋ１細胞の解凍と培養の開始

細胞: CHO-ｋ１　（RBRC-RCB0285, Riken Cell Bank）

Medium: GIBCO 11765-054　HAM's F12 ＋ 10% FBS

　　　　37 ºC で細胞急速解凍
　　　　　　│
　　　　Medium 5 ml
　　　　Cell 1ml
　　　　　　│ 6 ml
　　　　　　↓
　　　　遠心 1,000 rpm, 3 min
　　　　　　│
　　　　上清を捨て、細胞に Medium 10 ml　添加
　　　　　　│
　　　　細胞懸濁液を 10 mm dish に分注
　　　　　　│
　　　　37 ºC、5 % CO$_2$ で培養

「プロトコール 2」
CHO-k 1 細胞の継代

Medium: GIBCO 11765-054　HAM's F12 + 10% FBS

　　　37 ℃ の PBS で軽く細胞を洗浄
　　　　　　↓
　　　1 ml の Trypsin/EDTA 液を加える
　　　　　　↓
　　　37 ℃、1 min インキュベート
　　　　　　↓
　　　10 ml の Medium を添加
　　　　　　↓
　　　遠心 1,000 rpm, 3 min
　　　　　　↓
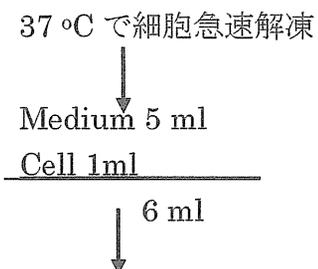　　　細胞に Medium 10 ml　添加
　　　　　　↓
　　　細胞カウント　1 x 10⁵ cells/ml に調整し、継代

「プロトコール 3」
CHO-k 1 細胞の解凍と培養の開始

細胞: CHO-k 1 − s c (suspension cell)(RBRC-RCB0403, Riken Cell Bank)

Medium: GIBCO 11765-054　HAM's F12 + 10% FBS

　　　37 ℃ で細胞急速解凍
　　　　　↓
　　Medium 5 ml
　　Cell 1ml
　　　　　　　　‾‾‾‾‾‾‾
　　　　↓ 6 ml
　　　　↓

遠心 1,000 rpm, 3 min

↓

細胞に Medium 20 ml　添加

↓

細胞懸濁液を 10 mm dish 2 枚に分注

↓

37 ℃、5 % CO_2 で培養

「プロトコール 4」
CHO-ｋ１－ｓｃ細胞の継代

37 ℃ の PBS で軽く細胞を洗浄

0.25%Trypsin/EDTA 液 1 ml を加える

↓

37 ℃、3min インキュベート

↓

5 ml の Medium を添加

↓

5 ml の Medium で共洗い

↓

遠心 1,000 rpm, 3 min

細胞に Medium 10 ml　添加

↓

細胞カウント　5 x 10^4 ～ 1 x 10^5 cells/ml に調整し、継代

第二節　CHO-k1 と CHO-k1-sc 細胞への rHRG 発現ベクターの導入と rHRG 発現の確認

　HEK293 細胞に用いた発現ベクターを用いて、接着系ならびに浮遊系の２種類の CHO 細胞を用いて、まず一過性の rHRG 発現について検討した。実験方法は、前章で記載した方法に準じた。

　結果と考察

　CHO-k1 と CHO-k1-sc 細胞いずれを用いた場合でも、HEK293 細胞を用いた場合と同様、一定量の組換え HRG を培養上清中に見出した。CHO 細胞で作製した HRG を、Ni-NTA アフィニティクロマトグラフィーで精製した。Ni-NTA アフィニティクロマトグラフィー上での挙動は、HEK293 細胞で作製した組換え HRG と略同様であった。今後、安定発現細胞株と大量培養系の確立を図っていかなければならない。

研究成果の刊行に関する一覧

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Wake H.,et al.	Histidine-rich glycoprotein prevents septic lethality through neutrophil regulation.				submitted
OkumaY.,et al.	Glycyrrhizin inhibits traumatic brain injury by reducing HMGB1-RAGE interaction.	*Neuropharmacology*			in press
Takahashi H.,et al.	Histamine inhibits high mobility group box 1-induced adhesion molecule expression on human monocytes.	*Eur J Pharmacol.*	718	305-13	2013
Nakamura Y.,et al.	Neuropathic pain in rats with a partial sciatic nerve ligation is alleviated by intravenous injection of monoclonal antibody to high mobility group box-1.	*PLOS ONE*	8(8)	e73640	2013
Takahashi HK.,et al.	Role of cell-cell interactions in high mobility group box 1 cytokine activity in human peripheral blood mononuclear cells and mouse splenocytes.	*Eur J Pharmacol.*	701	194-202	2013
西堀正洋	HMGB1 を標的とした治療	*日本臨牀*			印刷中
大熊佑 ほか	抗 HMGB1 抗体治療の可能性 －外傷性脳障害と神経因性疼痛に対する抗 HMGB1 抗体治療－	*日薬理誌*	143	5-9	2014
西堀正洋	DAMPs：HMGB1 の脳神経障害作用	*Thrombosis Medicine*	3(4)	5-11	2013
貞森裕 ほか	生体肝移植における high mobility group box-1 の動態解析	*日本消化器外科学会誌*	46(3)	232-5	2013

研究成果の刊行物・別刷

Immunopharmacology and inflammation

Histamine inhibits high mobility group box 1-induced adhesion molecule expression on human monocytes

CrossMark

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ARTICLE INFO

ABSTRACT

Cell–cell interaction through binding of adhesion molecules on monocytes to their ligands on T-cells plays roles in cytokine production and lymphocyte proliferation. High mobility group box 1 (HMGB1), an abundant and conserved nuclear protein, acts in the extracellular environment as a primary pro-inflammatory signal. HMGB1 induces expression of intercellular adhesion molecule (ICAM), B7.1, B7.2 and CD40 on monocytes, resulting in production of interferon (IFN)-γ and tumor necrosis factor (TNF)-α production and lymphocyte proliferation in human peripheral blood mononuclear cells (PBMCs). Histamine inhibits pro-inflammatory cytokine production via histamine H_2-receptors; however, it is not known whether histamine inhibits HMGB1 activity. This study was designed to study the inhibitory effect of histamine on HMGB1 activity. We examined the effect of histamine on HMGB1-induced expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes, production of IFN-γ and TNF-α and lymphocyte proliferation in PBMCs. Histamine inhibited HMGB1 activity in a concentration-dependent manner. The effects of histamine were partially ablated by the H_2-receptor antagonist, famotidine, and mimicked by the H_2/H_4-receptor agonists, dimaprit and 4-methylhistamine. Histamine induced cyclic adenosine monophosphate (cAMP) production in the presence and absence of HMGB1. The effects of histamine were reversed by the protein kinase A (PKA) inhibitor, H89, and mimicked by the membrane-permeable cAMP analog, dibutyryl cAMP (dbcAMP), and the adenylate cyclase activator, forskolin. These results together indicated that histamine inhibited HMGB1 activity

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1. Introduction

It has been known that the ubiquitous nuclear protein, high mobility group box 1 (HMGB1), modifies DNA structure to facilitate transcription, replication and repair (Bustin, 1999). An endogenous danger-associated molecular pattern protein (DAMP), which is released from stressed or injured cells, is the initial trigger for an inflammatory response. Recently, it has been reported that one of the most well-known DAMPs, namely the afore-mentioned HMGB1, is passively released from necrotic cells (Scaffidi et al., 2002) and secreted from stressed monocytes/macrophages (Gardella et al., 2002). Many studies have reported that extracellular HMGB1 has pro-inflammatory and immuno-

stimulatory properties and contributes to the pathogenesis of chronic inflammatory and autoimmune diseases, including hepatitis (Albayrak et al., 2010), rheumatoid arthritis (Kokkola et al., 2002), inflammatory bowel disease (McDonnell et al., 2011), acute lung inflammation (Abraham et al., 2000) and atherosclerosis (Porto et al., 2006).

Monocyte-derived costimulatory signals play roles in eliciting maximal T-cell proliferation, and cytokine production, lowering the concentration of antigen required for stimulation and promoting more sustained signaling from the T-cell receptor. The interaction of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40 on monocytes with their ligands on T-cells produces important costimulatory signals (Dustin and Springer, 1989; Greenfield et al., 1998). It has been reported that HMGB1 induces inflammatory responses, including maturation and migration of monocytes/macrophages (Rauvala and Rouhiainen, 2010), leading

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to activation of naïve T-cells in the promotion and induction of Th1 responses and to clonal expansion of antigen-specific T-cells (Messmer et al., 2004; Dumitriu et al., 2005). It is reported that HMGB1 induces production of tumor necrosis factor (TNF)-α, but not of interleukin (IL)-10 or IL-12, in normal human peripheral blood mononuclear cells (PBMCs) (Andersson et al., 2000). In a previous study, we found that HMGB1-induced pro-inflammatory cytokine production depended on an intimate cellular interplay between monocytes and T-cells in human PBMCs (Takahashi et al., 2013).

It has been reported that histamine modulates cytotoxic T-cell activity (Khan et al., 1989), NK-cell activity (Hellstrand et al., 1994) and cytokine production in human PBMCs (van der Pouw Kraan et al., 1998; Elenkov et al., 1998). Histamine activities depend on the stimulation of histamine H_1-, H_2-, H_3- and H_4-receptors (van der Pouw Kraan et al., 1998; Elenkov et al., 1998). Immunoregulatory effects of histamine are reported to depend on the stimulation of histamine H_2-receptors (van der Pouw Kraan et al., 1998; Elenkov et al., 1998; Hough, 2001). Histamine H_2-receptor stimulation induces the activation of adenylate cyclase and the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway in monocytes (Shayo et al., 1997). However, little is known about the effect of histamine on HMGB1-induced activity in monocytes.

In the present study, we examined the effect of histamine on HMGB1-induced expression of ICAM-1, B7.1, B7.2 and CD40, the production of interferon (IFN)-γ and TNF-α and lymphocyte proliferation in PBMCs.

2. Materials and methods

2.1. Reagents and drugs

Recombinant human (rh) HMGB1 was produced as described previously (Wake et al., 2009a). In brief, complementary DNA (cDNA) encoding full-length HMGB1 was amplified by polymerase chain reaction (PCR) from human microvascular endothelial cell cDNA. The PCR product was subcloned into a pGEX-6p-1 vector (GE Healthcare, Little Chalfont, England) to generate a glutathione S-transferase (GST) fusion protein. Sf9 insect cells (Invitrogen Life Technologies, NY) were transformed with the recombinant plasmid and incubated overnight at 37 °C in Overnight Express Instant TB Medium (Merck, San Diego, CA) to express recombinant GST-HMGB1. A Sf9 cell extract containing GST-HMGB1 fusion proteins was incubated with glutathione-Sepharose 4B for 1 h at room temperature. After washing, the gel bed was incubated with PreScission protease for 3 h at 4 °C. After a brief centrifugation, the supernatant containing HMGB1 with the GST tag removed was collected and purified by gel filtration chromatography using TSK-gel 3000SWXL (Tosoh, Tokyo, Japan). Purified rhHMGB1 protein was identified by Western blotting (Wake et al., 2009a) with a rat anti-human HMGB1 monoclonal Ab (mAb). The lipopolysaccharide (LPS) content of the purified rhHMGB1 was < 2.0 pg/μg protein.

Histamine dihydrochloride was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Dimaprit dihydrochloride and 4-methylhistamine dihydrochloride (4-MH) were gifts from Drs. WAM Duncan and DJ Durant (The Research Institute, Smith Kline and French Laboratories, Welwyn Garden City, Herts, UK). d-Chlorpheniramine maleate, ranitidine and famotidine were provided by Yoshitomi Pharmaceutical Co. Ltd. (Tokyo, Japan), Glaxo Japan (Tokyo, Japan) and Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan), respectively. Thioperamide hydrochloride was provided by Eisai Co. Ltd. (Tokyo, Japan). Dibutyryl cAMP (dbcAMP) and forskolin were purchased from

Wako Co., Ltd. (Tokyo, Japan). H89 was purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Isolation of PBMCs

Normal human PBMCs were obtained from ten healthy volunteers after acquiring Institutional Review Board approval (Okayama Univ. IRB No.106). Each 20–50 ml peripheral blood sample was withdrawn from a forearm vein, after which PBMCs were prepared and monocytes were separated from the PBMCs by counterflow centrifugal elutriation as previously described (Takahashi et al., 2002; Takahashi et al., 2003).

2.3. Flow cytometric analysis for adhesion molecule expression

For flow cytometric analysis, fluorescein isothiocyanate (FITC)-conjugated mouse IgG$_1$ mAb against human ICAM-1/CD54 and R-Phycoerythrin (PE)-conjugated anti-human CD14 mAb were purchased from DAKO (Glostrup, Denmark). FITC-conjugated mouse IgG$_1$ mAb against human B7.2 and CD40 were purchased from Pharmingen (San Diego, CA), and FITC-conjugated IgG$_1$ class-matched control was purchased from Sigma Chemical. FITC-conjugated mouse anti-mouse ICAM-1 mAb was purchased from DAKO. Changes in the expression of the human leukocyte antigens ICAM-1, B7.1, B7.2 and CD40 on monocytes (CD14) was examined by multi-color flow cytometry using a mixture of anti-CD14 Ab with anti-ICAM-1, anti-B7.1, anti-B7.2 or anti-CD40 Ab. PBMCs (4×10^6/ml) were incubated with 0.1–100 μg/ml HMGB1 and 0.1–100 μM histamine for 24 or 48 h at 37 °C in RPMI 1640 (Nissui Co. Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (FCS), 20 μg/ml kanamycin, 100 μg/ml streptomycin and penicillin, and 5×10^5/ml cultured cells were then prepared for flow cytometric analysis as previously described (Takahashi et al., 2002; Takahashi et al., 2003) and analyzed with a FACSCalibur (BD Biosciences, San Jose, CA). The data were processed using the CELL QUEST program.

2.4. Enzyme-linked immunosorbent assay

PBMCs (4×10^6/ml) were used for assessment of IFN-γ and TNF-α production. After incubation at 24 h at 37°C in a 5% CO_2/air mixture, cell-free supernatants were assayed for IFN-γ and TNF-α proteins by enzyme-linked immunosorbent assay (ELISA) using the multiple Abs sandwich principle (R&D Systems, Minneapolis, MN). The ELISA detection limit for both IFN-γ and TNF-α was 10 pg/ml.

2.5. Proliferation assay

PBMCs (4×10^6/ml) were treated with various reagents and incubated for 24 h at 37 °C in RPMI 1640 supplemented with 10% heat-inactivated FCS, 20 μg/ml kanamycin, 100 μg/ml streptomycin and penicillin, during which they were pulsed with [^{3}H]-thymidine (3.3 Ci/well) for the final 16 h. The cells were then dispensed into 96-well microplates, 200 μl/well, resulting in 1 μCi [^{3}H]-thymidine per well, and harvested with a Micro-Mate 196 Cell Harvester (Perkin Elmer Life Science Inc., Boston, MA, USA). Thymidine incorporation was measured with a Matrix 9600 β-counter (Perkin Elmer Life Science Inc., Yokohama, Japan).

2.6. Measurement of cAMP production in monocytes

Monocytes at 1×10^6 cells/ml were incubated at 37 °C in a 5% CO_2/air mixture under different conditions. When the effects of histamine receptor antagonists were examined, the antagonists

were added to the media 30 min before histamine addition. HMGB1 and histamine were simultaneously added to the media. After 24 h, cells (2×10^5 cells/200 µl/well) were supplemented with trichloroacetic acid to a final concentration of 5% and 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase, at 100 µM and frozen at $-80\ ^{\circ}$C. Frozen samples were subsequently sonicated and assayed for cAMP using a cAMP enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions, for which no acetylation procedures were performed. The results are expressed as the mean ± standard error of the mean (S.E.M.) for five donors.

2.7. Statistical analysis

Statistical significance was evaluated using ANOVA followed by a Dunnet's test. A probability value < 0.05 was considered to indicate statistical significance. Each data point was expressed as the mean ± S.E.M. of triplicate determinations from five donors.

3. Results

3.1. Effects of histamine on HMGB1-induced expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes, the production of IFN-γ and TNF-α and lymphocyte proliferation in PBMCs

HMGB1, at 10 µg/ml, significantly induced expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation at 16 h and, thereafter, up to 24 and 48 h (Takahashi et al., 2013). Adhesion molecule expression, cytokine production and lymphocyte proliferation increased at 1 µg HMGB1/ml and continued to increase up to 10 and 100 µg HMGB1/ml. As shown in Fig. 1, we observed the effects of histamine, at concentrations ranging from 0.1 to 100 µM, on expression of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and TNF-α and lymphocyte proliferation in the presence or absence of 10 µg/ml HMGB1 at 24 h. Histamine inhibited HMGB1 activities in a concentration-dependent manner. IC50 values for the inhibitory effect of histamine on expression of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and TNF-α and lymphocyte
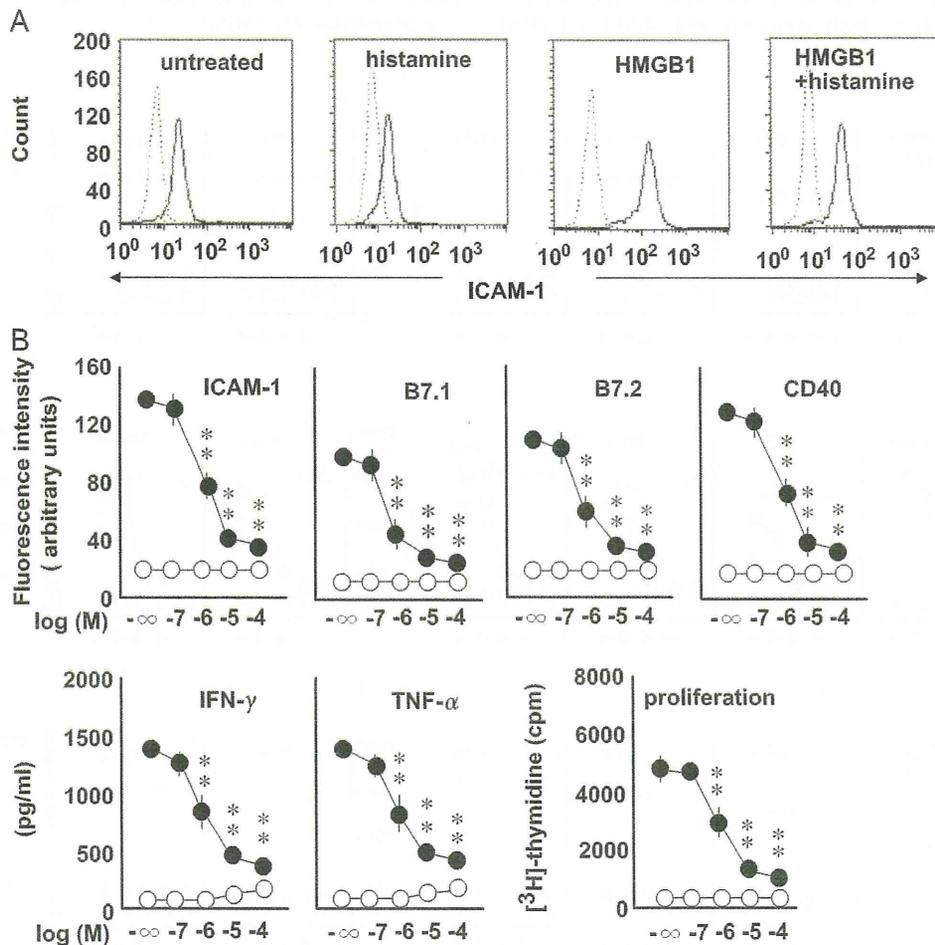

Fig. 1. Effects of histamine on HMGB1-induced expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes, production of IFN-γ and TNF-α and lymphocyte proliferation in human PBMCs. (A) PBMCs at 4×10^6 cells/ml were incubated with 10 µg/ml HMGB1 in the presence or absence of 100 µM histamine for 24 h. After incubation, ICAM-1 expression on monocytes (CD14) was determined by multi-color flow cytometry. Histogram of the expression of ICAM-1 on monocytes stained with anti-ICAM-1Ab (solid lines) or an IgG₁ classmatched control (dotted lines) was shown as representative data. (B) PBMCs were incubated with HMGB1 at 10 µg/ml and histamine at increasing concentrations from 0.1 to 100 µM for 24 h. The expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes was determined by flow cytometry. FITC-conjugated IgG₁ was used as an isotype-matched control Ab. IFN-γ and TNF-α concentration in conditioned media was determined by ELISA. Lymphocyte proliferation was determined by [³H]-thymidine uptake as described in Methods. Filled circles (●) represent effects of histamine in the presence of HMGB1, and open circles (○) represent that in the absence of HMGB1. The results are expressed as the mean ± S.E.M. of five donors with triplicate determinations. ** $P < 0.01$ compared with the value for HMGB1. When an error bar is within a symbol, the bar is omitted.

proliferation in the presence of HMGB1 were 0.8, 0.7, 1, 0.8, 1, 1 and 1 μM, respectively. In the absence of HMGB1, histamine induced the production of IFN-γ and TNF-α, but had no effect on adhesion molecule expression and lymphocyte proliferation.

### 3.2. Involvement of H_2-receptor in the actions of histamine

To determine the histamine receptor subtypes involved in mediating the effects of histamine on expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation in the presence of HMGB1, the activity of a histamine H_1-receptor antagonist, d-chlorpheniramine, a histamine H_2-receptor antagonist, famotidine, and a histamine H_3/H_4-receptor antagonist, thioperamide, at concentrations ranging from 0.1 to 100 μM on adhesion molecule expression, cytokine production and proliferation were examined in the presence of histamine at 10 μM (Fig. 2). Famotidine inhibited the action of histamine in a concentration-dependent manner, but d-chlorpheniramine and thioperamide had no effect. Another histamine H_2-receptor antagonist, ranitidine, exerted a substantially similar effect to famotidine (data not shown).

As shown in Fig. 3, the effects of histamine H_2/H_4-receptor agonists, dimaprit and 4-MH (Parsons et al., 1977), at concentrations ranging from 0.1 to 100 μM were determined in the presence of HMGB1 at 10 μg/ml. Both dimaprit and 4-MH inhibited
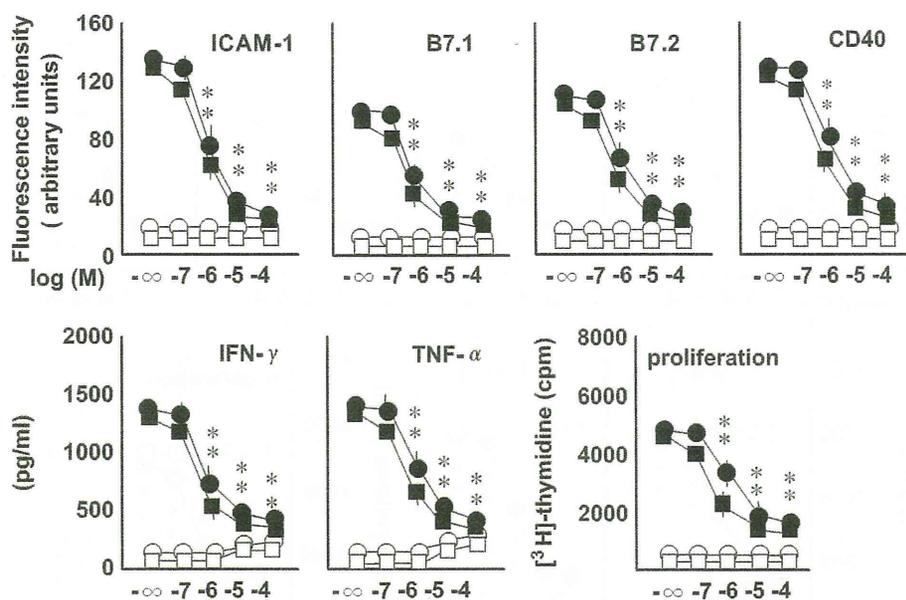
expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation in a concentration-dependent manner. The potency and efficacy of two agonists were quite similar to those of histamine in each response. Moreover, we found that a histamine H_1-agonist, 2-(2-pyridyl) ethylamine dihydrochloride (Durant et al., 1975), and a histamine H_3-agonist, (R)-α-methylhistamine dihydrochloride (Arrang et al., 1987), had no effect on adhesion molecule expression, cytokine production and lymphocyte proliferation induced by HMGB1 (data not shown).

3.3. Effects of histamine on the production of cAMP in monocytes in the presence or absence of HMGB1
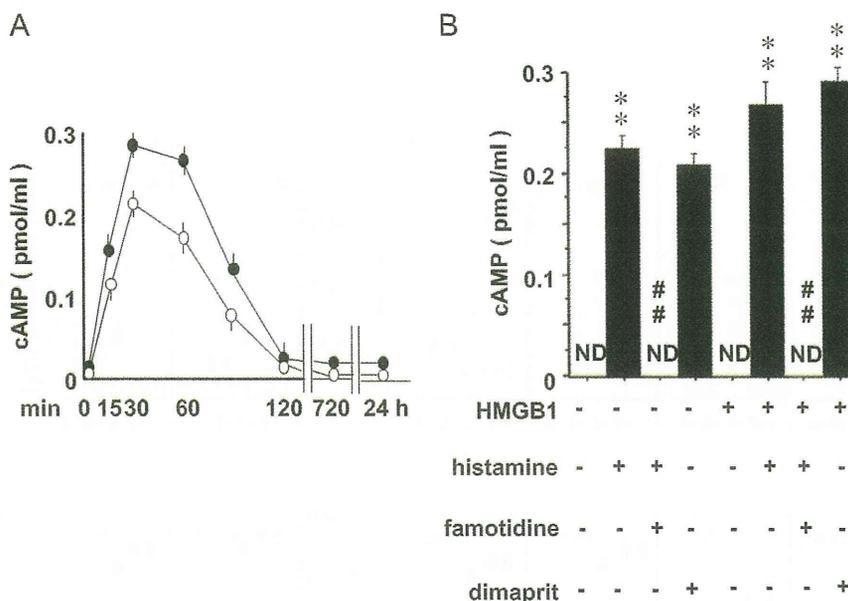
The effects of histamine at 100 μM on the production of intracellular cAMP in monocytes isolated from PBMCs in the presence or absence of 10 μg/ml HMGB1 were determined (Fig. 4). Histamine induced the production of cAMP in monocytes with a peak 30 min after stimulation. The presence of HMGB1 did not influence the production of cAMP induced by histamine. The histamine H_2-receptor antagonist, famotidine (at 100 μM) inhibited the effect of histamine on the production of cAMP (Fig. 4). Also, the histamine H_2/H_4-receptor agonist, dimaprit (at 100 μM) induced the production of cAMP (Fig. 4).
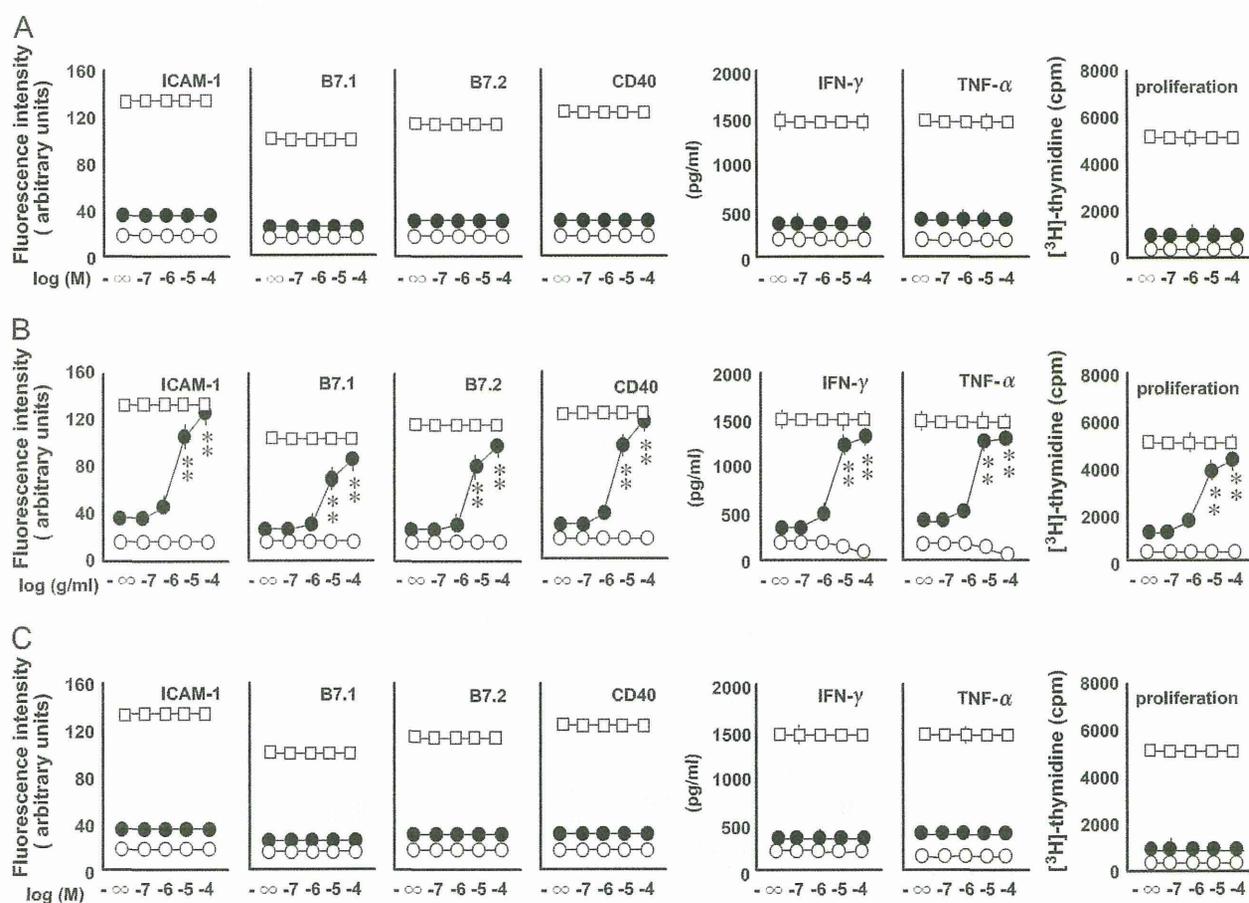
Fig. 2. Effects of histamine receptor antagonists on the actions of histamine. PBMCs at 4×10^6 cells/ml were incubated with different classes of histamine receptor antagonists, including d-chlorpheniramine (histamine H_1-receptor antagonist), famotidine (histamine H_2-receptor antagonist) and thioperamide (histamine H_3/H_4- receptor antagonist), at increasing concentrations from 0.1 to 100 μM in the presence or absence of 10 μM histamine for 24 h. The expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes was determined by flow cytometry. IFN-γ and TNF-α concentration in conditioned media was determined by ELISA. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake as described in Methods. Filled circles (●) represent the effects of antagonists on the actions of histamine in the presence of 10 μg/ml HMGB1. Open squares (□) represent the effect of antagonists in the presence of HMGB1 without histamine stimulation. Open circles (○) represent the effect of antagonists in the absence of histamine and HMGB1. The results are expressed as the mean ± S.E.M. of five donors with triplicate determinations. *P < 0.05, **P < 0.01 compared with the value for histamine. When an error bar is within a symbol, the bar is omitted.

Fig. 3. Effects of histamine receptor agonists on HMGB1-induced expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation and lymphocyte proliferation. PBMCs at 4×10^6 cells/ml were incubated with histamine H_2/H_4-receptor agonists, dimaprit and 4-MH at increasing concentrations from 0.1 to 100 μM in the presence of HMGB1 at 10 μg/ml for 24 h. The expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes was determined by flow cytometry, with IFN-γ and TNF-α levels in the conditioned media determined by ELISA. Lymphocyte proliferation was determined by [³H]-thymidine uptake as described in Methods. Filled circles (●) represent the effect of dimaprit in the presence of HMGB1, and open circles (○) represent effects in the absence of HMGB1. Filled squares (■) represent the effect of 4-MH in the presence of HMGB1, and open squares (□) represent effects in the absence of HMGB1. The results are expressed as the mean ± S.E.M. of five donors with triplicate determinations. **$P < 0.01$ compared with the value for HMGB1 alone. When an error bar is within a symbol, the bar is omitted.
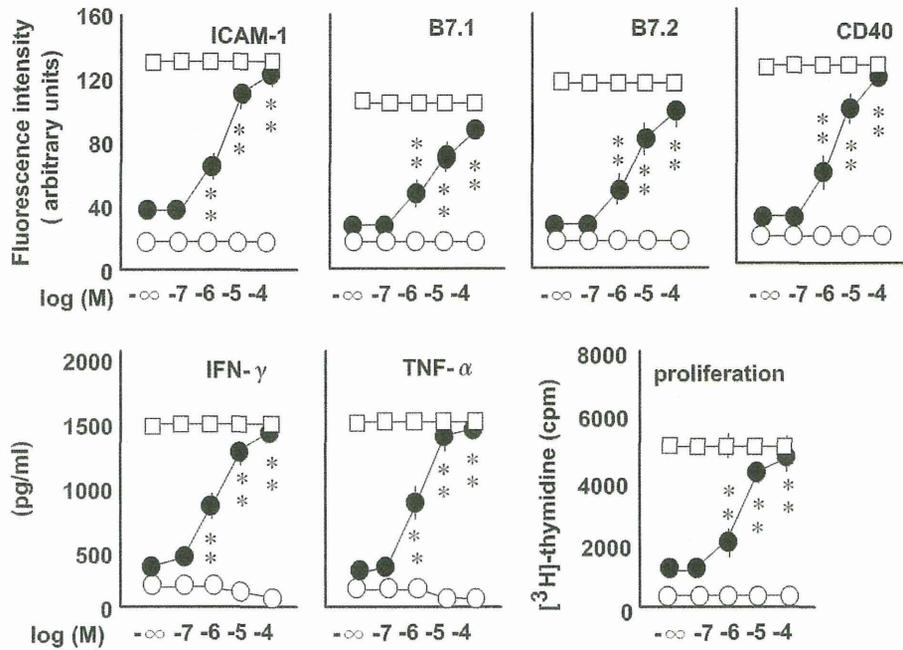

Fig. 4. Effects of histamine on the production of cAMP in monocytes in the presence or absence of HMGB1. (A) Monocytes at 1×10^6 cells/ml were incubated with histamine at 100 μM in the presence (filled circles; ●) and absence (open circles; ○) of HMGB1 at 10 μg/ml, and time course changes in the levels of cAMP in monocytes were determined at the indicated time points. (B) The effects of histamine and dimaprit at 100 μM in combination with famotidine on the production of cAMP were determined in the presence or absence of HMGB1 at 10 μg/ml. **$P < 0.01$ compared with the corresponding value in the absence of histamine. ##$P < 0.01$ compared with the corresponding value in the presence of histamine. The results are expressed as the mean ± S.E.M. of five donors with triplicate determinations. When an error bar was within a symbol, the bar was omitted. ND, not detected.

3.4. Involvement of cAMP in the action of histamine

To investigate the involvement of the cAMP/PKA pathway in the action of histamine, the effects of a PKA inhibitor, H89, at concentrations ranging from 0.1 to 100 μM on the action of histamine at 10 μM were determined (Fig. 5). In the absence of

histamine, the PKA inhibitor had no effect on adhesion molecule expression, cytokine production and lymphocyte proliferation. H89 reversed the inhibitory effect of histamine on expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation in the presence of 10 μg/ml HMGB1. As shown in Fig. 6, the effects of a membrane-permeable cAMP

Fig. 5. Effects of PKA inhibitor on the histamine-induced expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation. PBMCs at 4×10^6 cells/ml were incubated with a PKA inhibitor, H89, at increasing concentrations from 0.1 to 100 μM in the presence of HMGB1 at 10 μg/ml and histamine at 10 μM for 24 h. The expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes was determined by flow cytometry, with IFN-γ and TNF-α concentration in the conditioned media determined by ELISA. Lymphocyte proliferation was determined by [³H]-thymidine uptake as described in Methods. Filled circles (●) represent the effect of H89 on the actions of histamine in the presence of HMGB1. Open squares (□) represent those in the presence of HMGB1 without histamine stimulation. Open circles (○) represent the effect of H89 on the responses in the absence of both histamine and HMGB1. The results are expressed as the mean ± S.E.M. of triplicate findings from five donors. **$P < 0.01$ compared with the value in the presence of histamine and HMGB1. When an error bar is within a symbol, the bar is omitted.
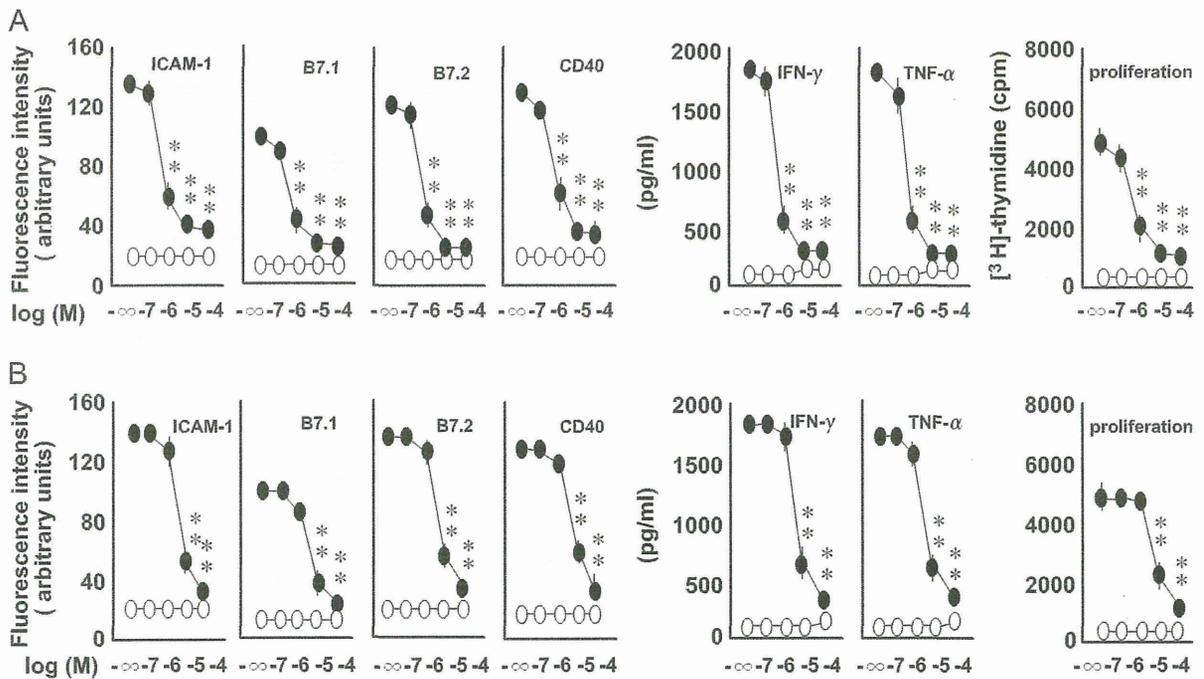
Fig. 6. Effects of forskolin and dbcAMP on HMGB1-induced expression of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation. PBMCs at 4×10^6 cells/ml were incubated with a cAMP analog, dbcAMP (A) and an adenylate cyclase activator, forskolin (B) at increasing concentrations from 0.01 to 10 μM in the presence of HMGB1 at 10 μg/ml for 24 h. The expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes was determined by flow cytometry, with IFN-γ and TNF-α concentration in the conditioned media determined by ELISA. Lymphocyte proliferation was determined by [³H]-thymidine uptake as described in Methods. Filled circles (●) represent the effect of histamine in the presence of HMGB1, and open circles (○) represent that in the absence of HMGB1. The results are expressed as the mean ± S.E.M. of five donors with triplicate determinations. **$P < 0.01$ compared with the value for HMGB1 alone. When an error bar is within a symbol, the bar is omitted.

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analog, dbcAMP, and an adenylate cyclase activator, forskolin, at concentrations ranging from 0.01 to 10 µM on expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes, production of IFN-γ and TNF-α and lymphocyte proliferation in PBMCs were examined. Both dbcAMP and forskolin inhibited HMGB1-induced adhesion molecule expression, cytokine production and lymphocyte proliferation in a concentration-dependent manner.

3.5. Effects of histamine on expression of CD14, TLR-2, TLR-4 and RAGE on monocytes

The receptor for advanced glycation end products (RAGE), toll-like receptor (TLR)-2 and TLR-4 are receptors for HMGB1 (Hori et al., 1995; Park et al., 2004; van Beijnum et al., 2008). In the previous study, the effect of incubation with 0.1–100 µg/ml HMGB1 on RAGE, TLR-2 and TLR-4 expression on monocytes in human PBMCs was determined after a 24 h incubation (Takahashi et al., 2013). HMGB1 increased RAGE expression in a concentration-dependent manner, but had no effect on TLR-2 or TLR-4 expression. The expression of

increased at 1 µg HMGB1/ml and continued to increase up to 10 and 100 µg HMGB1/ml. 10 µg HMGB1/ml enhanced the expression of CD14 (Fig. 7).

As shown in Fig. 7A, we observed the effects of histamine, at concentrations ranging from 0.1 to 100 µM, on expression of RAGE and CD14 in the presence or absence of 10 µg/ml HMGB1 at 24 h. Histamine inhibited expression of RAGE and CD14 in a concentration-dependent manner. The histamine H2-receptor antagonists, famotidine (Fig. 7B), as well as famotidine (data not shown), inhibited the action of histamine, while d-chlorpheniramine and thioperamide had no effect (data not shown). Moreover, histamine H2/H4-receptor agonists, dimaprit and 4-MH inhibited expression of RAGE and CD14 (Fig. 7C).

4. Discussion

A pro-inflammatory mediator HMGB1, which is secreted by activated monocytes/macrophages, is reported to induce inflammation
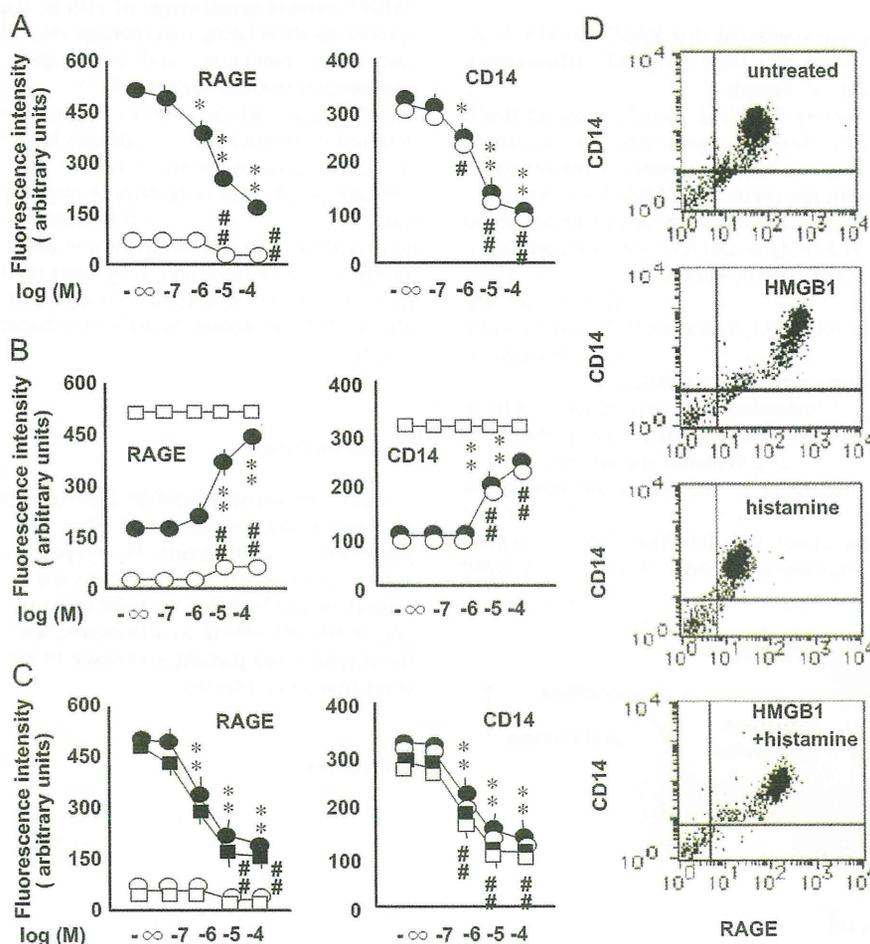
Fig. 7. Effects of histamine on HMGB1-induced expression of RAGE and CD14 in human PBMCs. (A) PBMCs at 4 × 10⁶ cells/ml were incubated with HMGB1 at 10 µg/ml and histamine at increasing concentrations from 0.1 to 100 µM for 24 h. Filled circles (●) represent the effect of histamine in the presence of HMGB1, and open circles (○) represent that in the absence of HMGB1. The results are expressed as the mean ± S.E.M. of five donors with triplicate determinations. *P < 0.05, **P < 0.01 compared with the value for HMGB1 alone. When an error bar is within a symbol, the bar is omitted. (B) PBMCs at 4 × 10⁶ cells/ml were incubated with histamine H2-receptor antagonist, famotidine at increasing concentrations from 0.1 to 100 µM in the presence or absence of 10 µM histamine for 24 h. Filled circles (●) represent the effects of antagonists on the actions of histamine in the presence of 10 µg/ml HMGB1. Open squares (□) represent the effect of antagonists in the presence of HMGB1 without histamine stimulation. Open circles (○) represent the effect of antagonists in the absence of histamine and HMGB1. ##P < 0.01, **P < 0.01 compared with the value for histamine. (C) PBMCs at 4 × 10⁶ cells/ml were incubated with histamine H2/H4-receptor agonists, dimaprit and 4-MH at increasing concentrations from 0.1 to 100 µM in the presence of HMGB1 at 10 µg/ml for 24 h. The expression of RAGE and CD14 on monocytes was determined by multi-color flow cytometry. Filled circles (●) represent the effect of dimaprit in the presence of HMGB1, and open circles (○) represent effects in the absence of HMGB1. Filled squares (■) represent the effect of 4-MH in the presence of HMGB1, and open squares (□) represent effects in the absence of HMGB1. ##P < 0.01 compared with the value for medium alone, and **P < 0.01 compared with the value for HMGB1. (D) PBMCs were incubated with or without 10 µg/ml HMGB1 and 100 µM histamine for 24 h and RAGE expression on monocytes was determined. The typical data was shown.

and injury (Wang et al., 1999). The expression of histamine has been observed in monocytes/macrophages, suggesting that histamine plays roles in the regulation of basic biological cell processes (Sasaguri and Tanimoto, 2004). In the present study, we clearly demonstrated, for the first time, that histamine inhibited HMGB1-induced adhesion molecule expression on monocytes, cytokine production and lymphocyte proliferation in human PBMCs (Fig. 1), indicating that HMGB1-induced inflammation and injury is modulated by an endogenous mediator, histamine. The effects of histamine were inhibited by the histamine H_2-receptor antagonist but not the histamine H_1-antagonist or the H_3/H_4-receptor antagonist (Fig. 2). The histamine $H_{2/4}$-receptor agonists mimicked the effects of histamine (Fig. 3). The IC50 values of histamine and histamine H_2/H_4-receptor agonists to prevent the up-regulation of adhesion molecule expression and cytokine production were consistent with the affinity of those agonists to typical histamine H_2-receptors (Johnson 1982; Elenkov et al., 1998; Kohka et al., 2000; Morichika et al., 2003; Takahashi et al., 2002), indicating that the inhibitory effects of histamine depended on the stimulation of histamine H_2-receptors. As shown in Figs. 4, 5 and 6, the findings, at least, suggested the involvement of the cAMP/PKA pathway in the effects of histamine.

In the previous study, we suggested that RAGE and TLR-4 are involved in the pathogenesis of a wide range of inflammatory disorders via recruitment of ligands (Takahashi et al., 2013). As shown in Fig. 7, histamine inhibited HMGB1-induced RAGE expression on monocytes through stimulation of histamine H_2-receptors, indicating that the inhibitory effect of histamine on HMGB1 activity depends on the regulation of RAGE expression.

We found a similar pattern of effects of histamine on advanced glycation end products (AGEs)-, LPS- and IL-18-induced activation of monocytes in human PBMCs via H_2-receptors (Morichika et al., 2003; Takahashi et al., 2002; Wake et al., 2009b). IL-18 is reported to induce expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes (Takahashi et al., 2002; Takahashi et al., 2003). While HMGB1 or AGEs do not induce production of IL-18 in PBMCs (Takahashi et al., 2013; Takahashi et al.,2009), histamine induces production of IL-18 via the histamine H_2-receptor and the cAMP/PKA pathway in monocytes (Takahashi et al., 2006). Whereas the amount of IL-18 production induced by histamine at 100 μM in the absence of HMGB1 was 2.5 ng/ml (Takahashi et al., 2006), that in the presence of HMGB1 was under the detection limit (10 pg/ml) (data not shown). Exogenously added IL-18 at 10 ng/ml

significantly inhibited the effects of histamine on HMGB1 activity (Takahashi et al.,2009). Moreover, it is reported that histamine alters the Th1/Th2 balance at the level of antigen presenting cells, Th1 and Th2 cells, or directly on effector cells (Elenkov et al., 1998). These results indicated an adverse effect of histamine. Thus, there may be a common pathway triggered by LPS, IL-18, AGEs and HMGB1 that is regulated by the histamine H_2-receptor-cAMP/PKA system. Further work is necessary to clarify this issue.

Macrophages/monocytes and T-cells play key roles in the immune responses of patients with inflammatory diseases; however, little is known regarding the mechanism underlying the effect of histamine on HMGB1 activities in human PBMCs. Intrahepatic recruitment of macrophages/monocytes and T-cells is reported to contribute to HMGB1-induced inflammation in an HBV-based mouse model of hepatitis (Sitia et al., 2007). In contrast, we found that endogenously produced histamine in Kupffer cells/macrophages plays a very important role in preventing an excessive innate immune response in endotoxin-induced fulminant hepatitis through the stimulation of histamine H_2-receptors (Yokoyama et al., 2004). Moreover, we found that HMGB1 exerted proatherogenic effects, augmenting lesion development by stimulating macrophage migration, modulating proinflammatory mediators, and encouraging the accumulation of immune and smooth muscle cells (Kanellakis et al.,2011). Histidine decarboxylase, which produces histamine from L-histidine, is detected in monocytes/macrophages located in the arterial intima in human atherosclerotic lesions (Higuchi et al., 2001). The resultant production of histamine may regulate vascular contraction (Tanimoto et al., 2007), indicating the modulatory effects of histamine on micro-inflammation in atherosclerotic intima. Thus, locally produced histamine may exert inhibitory influence on the activation of monocytes/macrophages and T-cells. Such a possibility should be evaluated by an in vivo hepatitis and atherosclerotic model.

5. Conclusions

Histamine inhibited HMGB1-induced expressions of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and TNF-α and lymphocyte proliferation via histamine H_2-receptors. Histamine inhibition of cellular interplay between monocytes and T-cells may reduce cytokine production and lymphocyte proliferation (Fig. 8). Through the inhibition of HMGB1 effects on monocytes, the stimulation of histamine H_2-receptors may partially contribute to regulating the development of inflammatory diseases.

Fig. 8. The effect of histamine on cellular interplay.

References

Abraham, E., Arcaroli, J., Carmody, A., Wang, H., Tracey, K.J., 2000. HMG-1 as a mediator of acute lung inflammation. Journal of Immunology 165, 2950–2954.
Albayrak, A., Uyanik, M.H., Cerrah, S., Altas, S., Dursun, H., Demir, M., Uslu, H., 2010. Is HMGB1 a new indirect marker for revealing fibrosis in chronic hepatitis and a new therapeutic target in treatment? Viral Immunology 23, 633–638.
Andersson, U., Wang, H., Palmblad, K., Aveberger, A.C., Bloom, O., Erlandsson-Harris, H., Janson, A., Kokkola, R., Zhang, M., Yang, H., Tracey, K.J., 2000. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. Journal of Experimental Medicine 192, 565–570.
Arrang, J.M., Garbarg, M., Lancelot, J.C., Lecomte, J.M., Pollard, H., Robba, M., Schunack, W., Schwartz, J.C., 1987. Highly potent and selective ligands for histamine H3-receptors. Nature (Lond) 327, 117–123.
Bustin, M., 1999. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Molecular and Cellular Biology 19, 5237–5246.
Dumitriu, I.E., Baruah, P., Valentinis, B., Voll, R.E., Herrmann, M., Nawroth, P.P., Arnold, B., Bianchi, M.E., Manfredi, A.A., Rovere-Querini, P., 2005. Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. Journal of Immunology 174, 7506–7515.
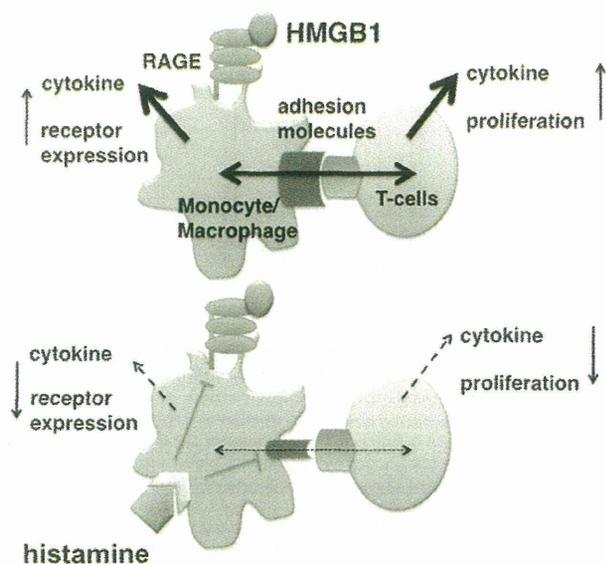
Durant, G.J., Ganellin, C.R., Parsons, M.E., 1975. Chemical differentiation of histamineH1- and H2-receptor agonists. Journal of Medicinal Chemistry 18, 905–913.

Dustin, M.L., Springer, T.A., 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature 341, 619–624.

Elenkov, I.J., Webster, E., Papanicolaou, D.A., Fleisher, T.A., Chrousos, G.P., Wilder, R. L., 1998. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. Journal of Immunology 161, 2586–2593.

Gardella, S., Andrei, C., Ferrera, D., Lotti, L.V., Torrisi, M.R., Bianchi, M.E., Rubartelli, A., 2002. The nuclear protein HMGB1 is secreted by monocytes via a nonclassical, vesicle-mediated secretory pathway. EMBO Reports 3, 995–1001.

Greenfield, E.A., Nguyen, K.A., Kuchroo, V.K., 1998. CD28/B7 costimulation: a review. Critical Reviews in Immunology 18, 389–418.

Hellstrand, K., Asea, A., Dahlgren, C., Hermodsson, S., 1994. Histaminergic regulation of NK cells. Role of monocyte-derived reactive oxygen metabolites. Journal of Immunology 153, 4940–4947.

Higuchi, S., Tanimoto, A., Arima, N., Xu, H., Murata, Y., Hamada, T., Makishima, K., Sasaguri, Y., 2001. Effects of histamine and interleukin-4 synthesized in arterial intima on phagocytosis by monocytes/macrophages in relation to atherosclerosis. FEBS Letters 505, 217–222.

Hori, O., Brett, J., Slattery, T., Cao, R., Zhang, J., Chen, J.X., Nagashima, M., Lundh, E.R., Vijay, S., Nitecki, D., Morser, J., Stern, D., Schmidt, A.M., 1995. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. Journal of Chemical Biology 270, 25752–25762.

Hough, L.B., 2001. Genomics meets histamine receptors: new subtypes, new receptors. Molecular Pharmacology 59, 415–419.

Johnson, C.L., 1982. Histamine receptors and cyclic nucleotides. In: Ganellin, C.R., Parsons, M.E. (Eds.), Pharmacology of Histamine Receptors. Wright PSG, Bristol, p. 146.

Kanellakis, P., Agrotis, A., Kyaw, T.S., Koulis, C., Ahrens, I., Mori, S., Takahashi, H.K., Liu, K., Peter, K., Nishibori, M., Bobik, A., 2011. High-mobility group box protein 1 neutralization reduces development of diet-induced atherosclerosis in apolipoprotein e-deficient mice. Arteriosclerosis, Thrombosis, and Vascular Biology 31, 313–319.

Khan, M.M., Keaney, K.M., Melmon, K.L., Clayberger, C., Krensky, A.M., 1989. Histamine regulates the generation of human cytolytic T lymphocytes. Cell Immunology 121, 60–73.

Kohka, H., Nishibori, M., Iwagaki, H., Nakaya, N., Yoshino, T., Kobashi, K., Saeki, K., Tanaka, N., Akagi, T., 2000. Histamine is a potent inducer of IL-18 and IFN-gamma in human peripheral blood mononuclear cells. Journal of Immunology 164, 6640–6646.

Kokkola, R., Sundberg, E., Ulfgren, A.K., Palmblad, K., Li, J., Wang, H., Ulloa, L., Yang, H., Yan, X.J., Furie, R., Chiorazzi, N., Tracey, K.J., Andersson, U., Harris, H.E., 2002. High mobility group box chromosomal protein 1: a novel proinflammatory mediator in synovitis. Arthritis and Rheumatism 46, 2598–2603.

McDonnell, M., Liang, Y., Noronha, A., Coukos, J., Kasper, D.L., Farraye, F.A., Ganley-Leal, L.M., 2011. Systemic toll-like receptor ligands modify B-cell responses in human inflammatory bowel disease. Inflammatory Bowel Disease 17, 298–307.

Messmer, D., Yang, H., Telusma, G., Knoll, F., Li, J., Messmer, B., Tracey, K.J., Chiorazzi, N., 2004. High mobility group box protein 1: an endogenous signal for dendritic cell maturation and Th1 polarization. Journal of Immunology 173, 307–313.

Morichika, T., Takahashi, H.K., Iwagaki, H., Yoshino, T., Tamura, R., Yokoyama, M., Mori, S., Akagi, T., Nishibori, M., Tanaka, N., 2003. Histamine inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in an intercellular adhesion molecule-1- and B7.1-dependent manner. Journal of Pharmacology and Experimental Therapeutics 304, 624–633.

Park, J.S., Svetkauskaite, D., He, Q., Kim, J.Y., Strassheim, D., Ishizaka, A., Abraham, E., 2004. Involvement of toll like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. Journal of Biological Chemistry 279, 7370–7377.

Porto, A., Palumbo, R., Pieroni, M., Aprigliano, G., Chiesa, R., Sanvito, F., Maseri, A., Bianchi, M.E., 2006. Smooth muscle cells in human atherosclerotic plaques secrete and proliferate in response to high mobility group box 1 protein. FASEB Journal 20, 2565–2566.

Rauvala, H., Rouhiainen, A., 2010. Physiological and pathophysiological outcomes of the interactions of HMGB1 with cell surface receptors. Biochimica et Biophysica Acta 1799, 164–170.

Sasaguri, Y., Tanimoto, A., 2004. Role of macrophage-derived histamine in atherosclerosis chronic participation in the inflammatory response. Journal of Atherosclerosis and Thrombosis 11, 122–130.

Shayo, C., Davio, C., Brodsky, A., Mladovan, A.G., Legnazzi, B.L., Rivera, E., Baldi, A., 1997. Histamine modulates the expression of c-fos through cyclic AMP production via the H2 receptor in the human promonocytic cell line U937. Molecular Pharmacology 51, 983–990.

Scaffidi, P., Misteli, T., Bianchi, M.E., 2002. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418, 191–195.

Sitia, G., Iannacone, M., Müller, S., Bianchi, M.E., Guidotti, L.G., 2007. Treatment with HMGB1 inhibitors diminishes CTL-induced liver disease in HBV transgenic mice. Journal of Leukocyte Biology 81, 100–107.

Takahashi, H.K., Iwagaki, H., Yoshino, T., Mori, S., Morichika, T., Itoh, H., Yokoyama, M., Kubo, S., Kondo, E., Akagi, T., Tanaka, N., Nishibori, M., 2002. Prostaglandin E (2) inhibits IL-18-induced ICAM-1 and B7.2 expression through EP2/EP4 receptors in human peripheral blood mononuclear cells. Journal of Immunology 168, 4446–4454.

Takahashi, H.K., Iwagaki, H., Tamura, R., Xue, D., Sano, M., Mori, S., Yoshino, T., Tanaka, N., Nishibori, M., 2003. Unique regulation profile of prostaglandin E1 on adhesion molecule expression and cytokine production in human peripheral blood mononuclear cells. Journal of Pharmacology and Experimental Therapeutics 307, 1188–1195.

Takahashi, H.K., Watanabe, T., Yokoyama, A., Iwagaki, H., Yoshino, T., Tanaka, N., Nishibori, M., 2006. Cimetidine induces interleukin-18 production through H2-agonist activity in monocytes. Molecular Pharmacology 70, 450–453.

Takahashi, H.K., Mori, S., Wake, H., Liu, K., Yoshino, T., Ohashi, K., Tanaka, N., Shikata, K., Makino, H., Nishibori, M., 2009. Advanced glycation end products subspecies-selectively induce adhesion molecule expression and cytokine production in human peripheral blood mononuclear cells. Journal of Pharmacology and Experimental Therapeutics 330, 89–98.

Takahashi, H.K., Sadamori, H., Liu, K., Wake, H., Mori, S., Yoshino, T., Yamamoto, Y., Yamamoto, H., Nishibori, M., 2013. Role of cell-cell interactions in high mobility group box 1 cytokine activity in human peripheral blood mononuclear cells and mouse splenocytes. European Journal of Pharmacology 701, 194–202.

Tanimoto, A., Wang, K.Y., Murata, Y., Kimura, S., Nomaguchi, M., Nakata, S., Tsutsui, M., Sasaguri, Y., 2007. Histamine upregulates the expression of inducible nitric oxide synthase in human intimal smooth muscle cells via histamine H1 receptor and NF-kappaB signaling pathway. Arteriosclerosis, Thrombosis, and Vascular Biology 27, 1556–1561.

van Beijnum, J.R., Buurman, W.A., Griffioen, A.W., 2008. Convergence and amplification of toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) signaling pathways via high mobility group B1 (HMGB1). Angiogenesis 11, 91–99.

van der Pouw Kraan, T.C., Snijders, A., Boeije, L.C., de Groot, E.R., Alewijnse, A.E., Leurs, R., Aarden, L.A., 1998. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. Journal of Clinical Investigation 102, 1866–1877.

Wake, H., Mori, S., Liu, K., Takahashi, H.K., Nishibori, M., 2009a. High mobility group box 1 complexed with heparin induced angiogenesis in matrigel plug assay. Acta Medica Okayama 63, 249–262.

Wake, H., Takahashi, H.K., Mori, S., Liu, K., Yoshino, T., Nishibori, M., 2009b. Histamine inhibits advanced glycation end products-induced adhesion molecule expression on human monocytes. Journal of Pharmacology and Experimental Therapeutics 330, 826–833.

Wang, H., Bloom, O., Zhang, M., Vishnubhakat, J.M., Ombrellino, M., Che, J., Frazier, A., Yang, H., Ivanova, S., Borovikova, L., Manogue, K.R., Faist, E., Abraham, E., Andersson, J., Andersson, U., Molina, P.E., Abumrad, N.N., Sama, A., Tracey, K.J., 1999. HMG-1 as a late mediator of endotoxin lethality in mice. Science 285, 248–251.

Yokoyama, M., Yokoyama, A., Mori, S., Takahashi, H.K., Yoshino, T., Watanabe, T., Watanabe, T., Ohtsu, H., Nishibori, M., 2004. Inducible histamine protects mice from P. acnes-primed and LPS-induced hepatitis through H2-receptor stimulation. Gastroenterology 27, 892–902.