図１　ネフローゼモデル実験群

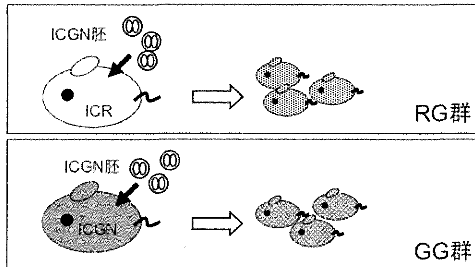


図２　糸球体半月体の出現頻度

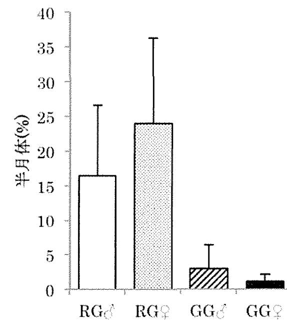


図３　ネフローゼマウス腎の免疫組織化学解析

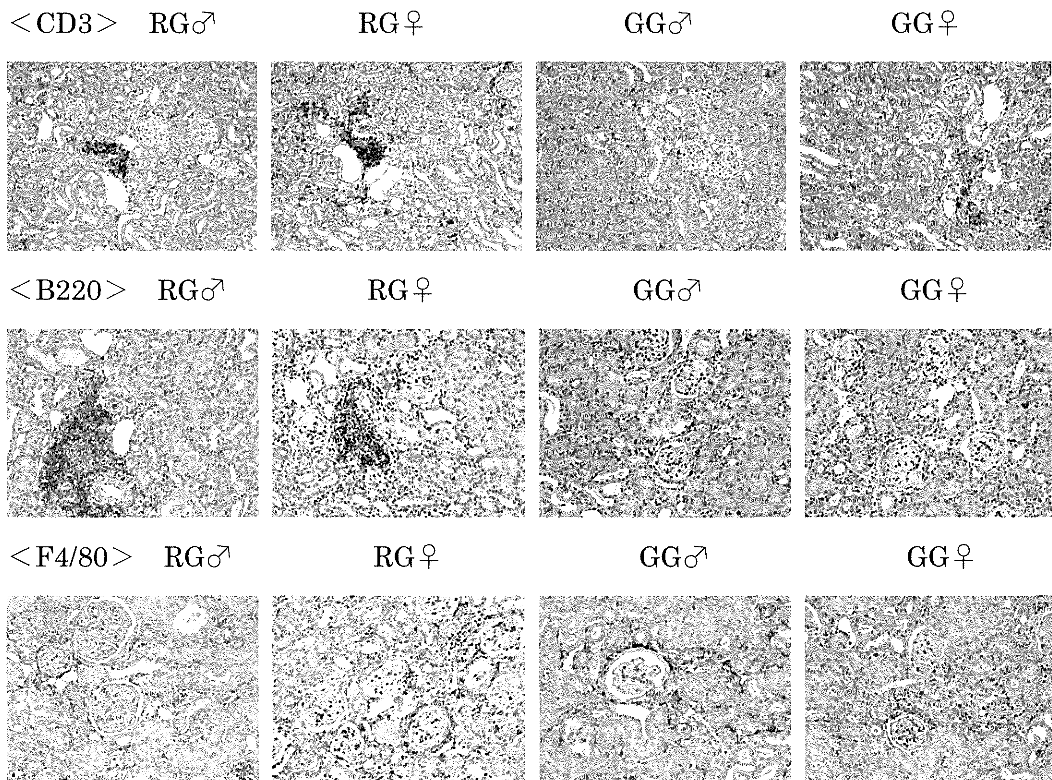


図４　ネフローゼマウス腎の Real Time PCR 解析結果

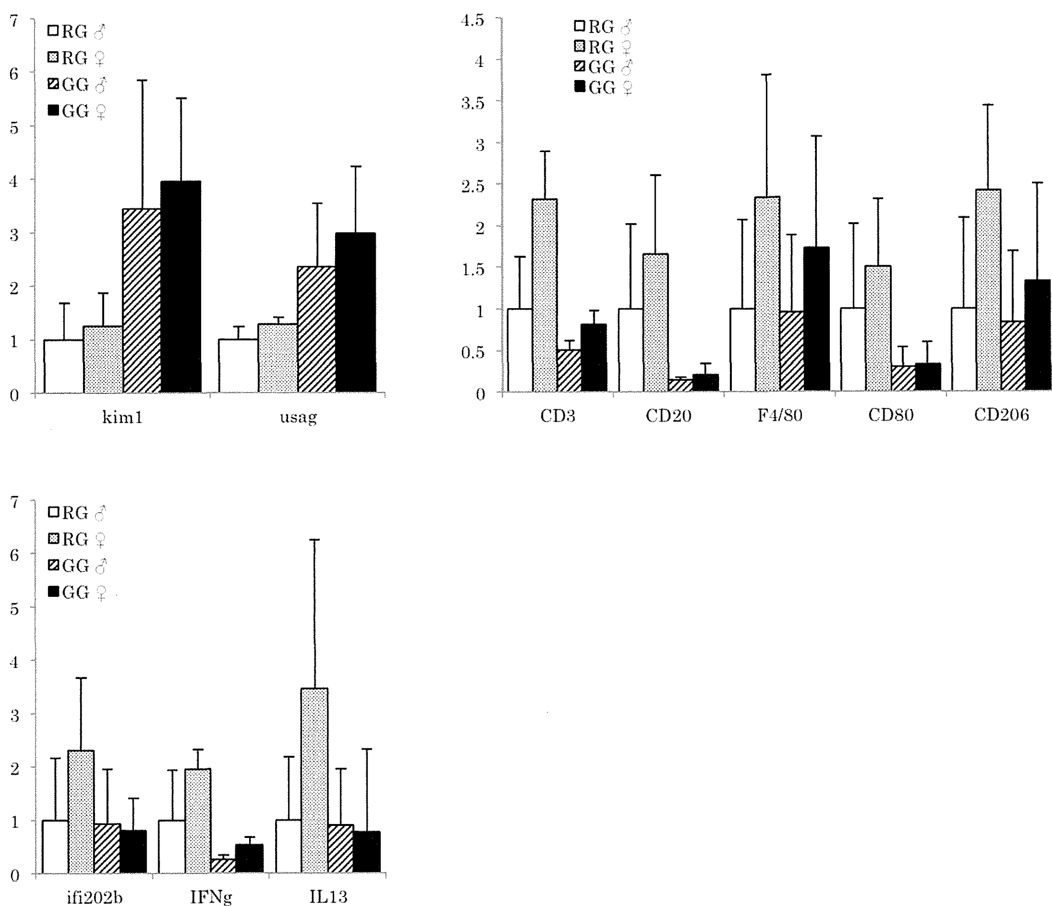
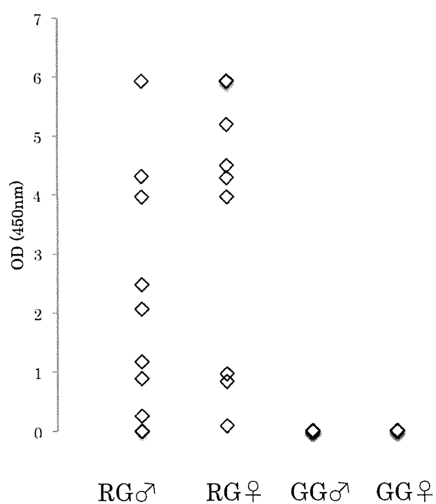


図５　ネフローゼマウス血清の dsDNA 抗体価



厚生労働科学研究費補助金(創薬基盤推進研究事業)
分担研究報告書

周産期疾患の解析と繁殖技術の開発のためのカニクイザル MHC class-Iのタイピング解析

分担研究者 高橋一朗 (医薬基盤研究所 難病・疾患資源研究部)

研究要旨

SIV 感染に対して感受性があるか或いは抵抗性かは、MHC の class I が重要な働きを持っていると考えられている。 そこで基盤研霊長類センターで飼育管理されているカニクイザル個体より作成された株化 B 細胞株で発現している MHC class I A ローカス、B ローカスからの発現タイプと SIV 感染感受性の相関を調べた。

カニクイザル5個体より作成された B 細胞株で発現している MHC class I についてシークエンス法で同定した。しかし感染抵抗性を付加する分子の同定はできなかった。

複数個のアレルの関与あるいは、複数個のローカスの関与が強く疑われた。

A. 研究目的

霊長類センターで飼育管理しているカニクイザルコロニーの遺伝学的解析を行うことにより、コロニーの特色を把握し実験用カニクイザルの高度化を図り資源としての価値を高める。またヒトの疾病モデルとしてカニクイザルを使用するための基礎データとして、MHC 遺伝子の発現情報を蓄積する。

アカゲザルでのMHC class-I のタイピングおよびゲノム解析した結果の整備がすすんでいるが、カニクイザルについては、体系的に進んでいない。そこで感染症に対する防御を担っているMHC class I を調べることで、実験動物のカニクイザルの資源の高度化を図ることを目的とする。

B. 研究方法

カニクイザル個体より採血された血液よりB細胞を株化した細胞を作製した。

カニクイザル B 細胞株については SIV 感染前に作成され、作製後に SIV 感染実験を行い感染の成立、感染の非成立が行われた。これらB細胞株5株より発現しているRNAを抽出しMHC 特異的なプライマーで増幅後クローニングし約10クローンをシークエンスして塩基配列を比較した。

その得られた結果よりエクソン2から3の部分について比較して MHC のタイプを同定した。

C. 研究結果

作製されたB 細胞株をA,B,C,D,E とすると、そのうち2株 A,B が SIV 感染抵抗個体由来の細胞である。5株の B 細胞株より発現して

いるMHC class I Aローカス、Bローカスは、複数発現しており、これらの塩基配列を決定し、クラスタ解析を行った。SIV ウィルス感染抵抗性個体と感染感受性個体との MHC クラス I の特異的なタイプは特定できなかった。
（図　参照）

D. 考察

霊長類の実験動物として最もポピュラーであるカニクイザルについて、MHC のタイピングは非常に重要な情報であるが個体で発現しているMHCの情報はクローンの塩基配列情報の登録は多くなされているが、そのMHC分子の発現により知られている感染症ウィルス感染に対して感受性かあるいは抵抗性かは報告がない。これについては多くの個体で発現している MHC を解析し感染抵抗性の固体の数を増やして解析する必要がある。
それとともに複数のアレルの関与も考えられ2個体からの結果からは判定できないが、少なくとも2個体では違うアレルが働いていると思われる。

E. 結論

MHC class I 抗原は感染症の抵抗性を付与することが知られており、あらかじめ抵抗性を付与するMHC class I分子の発現を調べることができたとしたら実験動物としてのカニクイザル資源の高度化に役立つと考えられる。

F. 健康危険情報

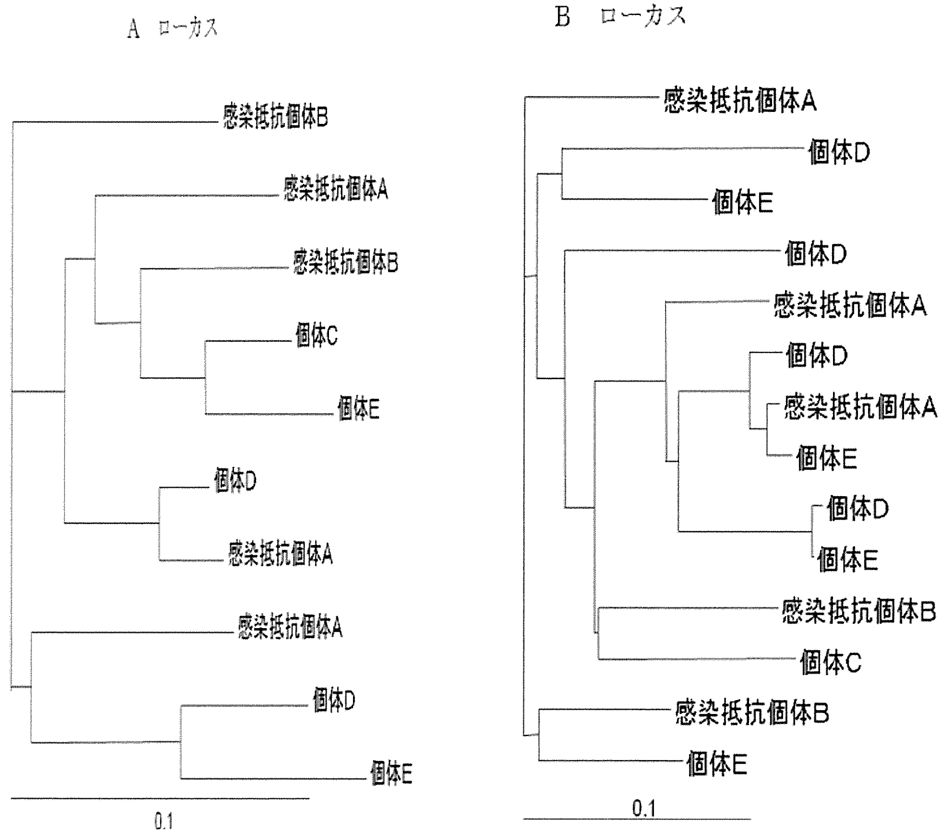
特になし。

G. 研究発表

なし。

H. 知的財産権の出願・登録状況
（予定を含む。）

なし。



図： MHC class I 遺伝子エキソン2，3部分のクラスタ解析

研究成果の刊行に関する一覧表

（平成２４年度）

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
山海　直(分担翻訳)	犬と猫の重篤症例における臓器不全	原著者：Frederic P. Gaschen、監訳者：金山喜一、鯉江　洋	サンダース・ベテリナリー・クリニクスシリーズ Vol. 7 No. 3	インダース/エルゼビア・ジャパン		印刷中	
山海　直(分担翻訳)	犬と猫の慢性腸疾患　－病態生理・診断・治療の最新情報－	原著者：Frederic P. Gaschen、監訳者：金山喜一、鯉江　洋	サンダース・ベテリナリー・クリニクスシリーズ Vol. 7 No. 3	インダース/エルゼビア・ジャパン		2012	

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
Yoshhida, T., Omatsu, T., Saito, A., Katakai, Y., Iwasaki, Y., Kurosawa, T., Hamano, M., Higashimo, A., Nakamura, S.., Takasaki, T., Yasutomi, Y., Kurane, I. and Akari, H.	Dynamics of cellular immune responses in the acute phase of dengue virus infection.	Archiv. Virol.			印刷中
Tougan,T., Aoshi,T., Coban,C., Katakai,Y., Kai,C., Yasutomi,Y., Ishii,KJ. and Horii,T.	TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in nonhuman primate models.	Hum.Vac.Immunother			印刷中

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Karamatsu,K., Matsuo,K., Inada,H., Tsujimura,Y., Shiogama,Y., Matsubara,A., Kawano,M. and Yasutomi,Y.	Single systemic administration of Ag85B of mycobacteria DNA inhibits allergic airway inflammation in a mouse model of asthma.	J Asthma Allergy	5	71-79	2012
Nomaguchi,M., Yokoyama,M., Kono,K., Nakayama,E.E., Shioda T., Saito,A., Akari,H., Yasutomi,Y., Matano,T., Sato,H. and Adachi,A.	Gag-CA Q110D mutation elicits TRIM5-independent enhancement of HIV-1mt replication in macaque cells.	Microve. Infect			印刷中
Yoshida,T., Omatsu,T., Saito,A., Katakai,Y., Iwasaki,Y., Iijima,S., Kurosawa,T., Hamano,M., Nakamura,S., Takasaki,T., Yasutomi,Y., Kurane.,I Akari,H.	CD16 positive natural killer cells play a limited role against primary dengue virus infection in tamarins	Archives Virol	15	363-368	2012
Tajiri,K., Imanaka-Yoshida,K., Matsubara,A., Tsujimura,Y., Hiroe,M., Naka,T.,Shimojo,N., Sakai,S., Aonuma,K. and Yasutomi,Y.	Suppressor of cytokine signaling 1 (SOCS1) DNA administration inhibits inflammatory and pathogenic responses in autoimmune myocarditis.	J.Immunol	189	2043-2053	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Uchdida,A., Sasaguri,H., Kimura,N., Tajiri,M., Ohkubo,T., Ono,F., Sakaue,F., Kanai,K., Hirai,T., Sano,T., Shibuya,K., Kobayashi,M., Yamamoto,M., Yokota,S.., Kuboddera,T., Tomori,M., Sakaki,K., Enomoto,M., Hirai,Y., Kumagai,J., Yasutomi,Y., Mochizuki,H., Kuwabara,S., Uchihara,T., Mizusawa,H. and Yokakota,T.	Non-human primate model of ALS with cytoplasmic mislocalization of TDP-43.	Brain	135	833-846	2012
Saito,A., Kono,K., Nomaguchi,M., Yasutomi,Y., Adachi,A., Shioda,T., Akari,H. and Nakayama,E. E.	Geographical genetic and functional diversity of antiretroviral host factor TRIMCyp in cynomolgus macaque (Macaca fascicularis).	J.Gen.Virol.	93	594-602	2012
Higashino,A., Sakate,R., Kameoka,Y., Takahashi,I., Hirata,M., Tanuma,R., Masui,T., Yasutomi,Y. and Osada,N.	Whole-genome sequencing and analysis of the Malaysian cynomolgus macaque (Macaca fascicularis) genome.	Genome Biol.	Epub		2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tachibana,S., Sullivan,SA., Kawai,S., Nakamura,S., Goto,N., Arisue,N., Palacpac,NMQ., Honma,H., Yagi,M., Tougan,T., Katakai,Y., Kaneko,O., Mita,T., Kita,K., Yasutomi,Y., Kim,HR., Sutton,PL., Shakhbatyan,R., Horii,T., Yasunaga,T., Bamwell,JW., Escalante,AA., Carlton,JM. And Tanabe,K.	Plasmodium cynomolgi genome sequences provide insight into Plasmodium vivax and the monkey malaria clade.	Nature Genetics	44	1051-1055	2012
Junko Otsuki, Yasushi Nagai, Alex Lopata, Kazuyoshi Chiba, Yasmin Lubna, Tadashi Sankai	Symmetrical division of mouse oocytes during meiotic maturation can lead to the development of twin embryos that amalgamate to form a chimeric hermaphrodite	Hum. Reprod.	27	380-387	2012
Juri Morichika, Chizuru Iwatani, Hideaki Tsuchiya, Shinichiro Nakamura, Tadashi Sankai, Ryuzo Torii	Triplet pregnancy in a cynomolgus monkey (*Macaca fascicularis*) after double embryo transfer	Comp. Med.	62	1-4	2012
山海　直	早期妊娠診断と胎児の雌雄判定技術の開発研究に携わって(巻頭総説)	Letters of Society of Primate Diseases and Pathology	3	2-3	2012
Shimozawa N, Ono R, Shimada M, Shibata H, Takahashi I, Inada H, Takada T, Nosaka T, Yasutomi Y.	Cynomolgus monkey induced pluripotent stem cells established by using exogenous genes derived from the same monkey species.	Differentation			In press

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Uebi T, Itoh Y, Hatano O, Kumagai A, Sanosaka M, Sasaki T, Sasagawa S, Doi J, Tatsumi K, Mitamura K, Morii E, Aozasa K, Kawamura T, Okumura M, Nakae J, Takikawa H, Fukusato T, Koura M, Nish M, Hamsten A, Silveira A, Bertorello AM, Kitagawa K, Nagaoka Y, Kawahara H, Tomonaga T, Naka T, Ikegawa S, Tsumaki N, Matsuda J, Takemori H.	Involvement of SIK3 in glucose and lipid homeostasis in mice.	PLoS One.	7(5)	e37803	2012
Suzuki Y, Ichinomiya S, Kurosawa M, Matsuda J, Ogawa S, Iida M, Kubo T, Tabe M, Itoh M, Higaki K, Nanba E, Ohno K.	Therapeutic Chaperone Effect of N-Octyl 4-Epi-β-Valienamine on Murine GM1-Gangliosidosis.	Mol Genet Metab.	106	92-98	2012
Iwata Y, Suzuki O, and Watanabe S.	Decreased surface sialic acid content is a sensitive indicator of muscle damage. DOI: 10.1002/mus.23632.	Muscle and Nerve	47(3)	372-378	2013
Kinehara M, Kawamura S, Tateyama D, Suga M, Matsumura H, Mimura S, Hirayama N, Hirata M, Uchio-Yamada K, Kohara A, Yanagihara K, Furue MK.	Protein kinase C regulates human pluripotent stem cell self-renewal.	PLoS One	e54122	118-127	2013

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takeda S, Sato N, Uchio-Yamada K, Yu H, Moriguchi A, Rakugi H, Morishita R.	Oral glucose loading modulates plasma β-amyloid level in alzheimer's disease patients: potential diagnostic method for Alzheimer's disease.	Dement Geriatr Cogn Disord.	34(1)	25-30	2012
山本 美江、内尾-山田 こずえ、高野 薫、小倉 淳郎、浅野 敏彦、中川 雅郎	「ICR 系マウスの遺伝子資源としての有用性」	岡山実験動物研究会会報	第 28 号	59-62	2012

Arch Virol
DOI 10.1007/s00705-013-1618-6

ORIGINAL ARTICLE

Dynamics of cellular immune responses in the acute phase of dengue virus infection

Tomoyuki Yoshida · Tsutomu Omatsu · Akatsuki Saito · Yuko Katakai · Yuki Iwasaki · Terue Kurosawa · Masataka Hamano · Atsunori Higashino · Shinichiro Nakamura · Tomohiko Takasaki · Yasuhiro Yasutomi · Ichiro Kurane · Hirofumi Akari

Received: 13 June 2012 / Accepted: 12 December 2012
© Springer-Verlag Wien 2013

Abstract In this study, we examined the dynamics of cellular immune responses in the acute phase of dengue virus (DENV) infection in a marmoset model. Here, we found that DENV infection in marmosets greatly induced responses of CD4/CD8 central memory T and NKT cells. Interestingly, the strength of the immune response was greater in animals infected with a dengue fever strain than in those infected with a dengue hemorrhagic fever strain of DENV. In contrast, when animals were re-challenged with the same DENV strain used for primary infection, the neutralizing antibody induced appeared to play a critical role in sterilizing inhibition against viral replication, resulting in strong but delayed responses of CD4/CD8 central memory T and NKT cells. The results in this study may help to better understand the dynamics of cellular and humoral immune responses in the control of DENV infection.

T. Yoshida and T. Omatsu contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00705-013-1618-6) contains supplementary material, which is available to authorized users.

T. Yoshida · Y. Iwasaki · T. Kurosawa · M. Hamano · Y. Yasutomi · H. Akari
Tsukuba Primate Research Center, National Institute of Biomedical Innovation, 1-1 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan

T. Yoshida (✉) · A. Saito · A. Higashino · H. Akari (✉)
Center for Human Evolution Modeling Research, Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan
e-mail: yoshida.tomoyuki.4w@kyoto-u.ac.jp

H. Akari
e-mail: akari.hirofumi.5z@kyoto-u.ac.jp

Introduction

Dengue virus (DENV) causes the most prevalent arthropod-borne viral infections in the world [29]. Infection with one of the four serotypes of DENV can lead to dengue fever (DF) and sometimes to fatal dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [12]. The serious diseases are more likely to develop after secondary infection with a serotype of DENV that is different from that of the primary infection. Infection with DENV induces a high-titered neutralizing antibody response that can provide long-term immunity to the homologous DENV serotype, while the effect of the antibody on the heterologous serotypes is transient [22]. On the other hand, enhanced pathogenicity after secondary DENV infection appears to be explained by antibody-dependent enhancement (ADE). Mouse and monkey experiments have shown that subneutralizing levels of DENV-specific antibodies actually enhance infection [1, 6, 11]. Thus, the development of an effective tetravalent dengue vaccine is considered to be an important public-health priority. Recently, several DENV vaccine candidates have undergone clinical trials, and most of them target the induction of neutralizing antibodies [20].

T. Omatsu · T. Takasaki · I. Kurane
Department of Virology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

Y. Katakai
Corporation for Production and Research of Laboratory Primates, 1-1 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan

S. Nakamura
Research Center for Animal Life Science, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga 520-2192, Japan

Published online: 05 February 2013

Research of the long-term immune response in humans has provided several interesting parallels to the data. It was reported that complete cross-protective immunity from heterologous challenge was induced in individuals 1–2 months after a primary DENV infection, with partial immunity present up to 9 months, resulting in a milder disease of shorter duration on reinfection, and that complete serotype-specific immunity against symptomatic dengue was observed up to 18 months postinfection [30]. Guzman and Sierra have previously recorded the long-term presence of both DENV-specific antibodies and T cells up to 20 years after natural infections [10, 31]. Of note, increased T cell activation is reportedly associated with severe dengue disease [7, 8]. Thus, the balance between humoral and cellular immunity may be important in the control of dengue diseases.

However, the details regarding the implication of humoral and cellular immunity in controlling DENV infection remain to be elucidated. Previously, passive transfer of either monoclonal or polyclonal antibodies was shown to protect against homologous DENV challenge [13, 15, 16]. It was also reported that neutralizing antibodies played a greater role than cytotoxic T lymphocyte (CTL) responses in heterologous protection against secondary DENV infection *in vivo* in IFN-$\alpha/\beta R^{-/-}$ and IFN$\gamma R^{-/-}$ mouse models [18]. Moreover, $CD4^+$ T cell depletion did not affect the DENV-specific IgG or IgM Ab titers or their neutralizing activity in the IFN$\gamma R^{-/-}$ mouse model [36]. On the other hand, there are several reports showing that cellular immunity rather than humoral immunity plays an important role in the clearance of DENV. For example, in adoptive transfer experiments, although cross-reactive DENV-1-specific $CD8^+$ T cells did not mediate protection against a lethal DENV-2 infection, adoptive transfer of $CD4^+$ T cells alone mediated protection and delayed mortality in IFN-$\alpha/\beta R^{-/-}$ and IFN$\gamma R^{-/-}$ mouse models [39]. It has also been demonstrated that $CD8^+$ T lymphocytes have a direct role in protection against DENV challenge in the IFN-$\alpha/\beta R^{-/-}$ mouse model of DENV infection by depleting $CD8^+$ T cells [35]. In addition, previous data from adoptive-transfer experiments in BALB/c mice showed that cross-reactive memory $CD8^+$ T cells were preferentially activated by the secondary DENV infection, resulting in augmented IFN-γ and tumor necrosis factor-α (TNF-α) responses, and this effect was serotype-dependent [2, 3]. Although it has previously been suggested that inducing neutralizing antibodies against DENV may play an important role in controlling DENV infection, CTLs are also proposed to contribute to clearance during primary DENV infection and to pathogenesis during secondary heterologous infection in the BALB/c mouse model [4].

Why did the mouse models of DENV infection show inconsistent results *in vivo*? One of the reasons could be that these results were obtained mainly from genetically manipulated mice such as IFN-$\alpha/\beta R^{-/-}$ and IFN$\gamma R^{-/-}$ mice. Moreover, these mice were inoculated with 10^9–10^{10} genome equivalents (GE) of DENV [27, 35, 36], which were likely in large excess compared with the 10^4-10^5 GE of DENV injected into humans by a mosquito [19]. In addition, the efficiency of DENV replication in wild mice *in vivo* is very low compared to that in humans [35].

Recently, novel non-human primate models of DENV infection using rhesus macaques as well as marmosets and tamarins have been developed [24–26, 38]. An intravenous challenge of rhesus macaques with a high dose of virus inoculum (1×10^7 GE) of DENV-2 resulted in readily visible hemorrhaging, which is one of the cardinal symptoms of human DHF [26]. It was also shown that the cellular immune response was activated due to expression of IFN-γ, TNF-α, and macrophage inflammatory protein-1 β in $CD4^+$ and $CD8^+$ T cells during primary DENV infection in rhesus macaques [20]. On the other hand, in the marmoset model of DENV infection, we observed high levels of viremia (10^5–10^7 GE/ml) after subcutaneous inoculation with 10^4–10^5 plaque-forming units (PFU) of DENV-2. Moreover, we demonstrated that DENV-specific IgM and IgG were consistently detected and that the DENV-2 genome was not detected in any of these marmosets inoculated with the same DENV-2 strain used in the primary infection [24]. It is notable that while neutralizing antibody titers were at levels of 1:20-1:80 before the re-challenge inoculation, the titers increased up to 1:160-1:640 after the re-challenge inoculation [24]. These results suggested that the secondary infection with DENV-2 induced a protective humoral immunity to DENV-2 and that DENV-infected marmoset models may be useful in order to analyze the relationship between DENV replication and the dynamics of adaptive immune responses *in vivo*.

Taking these findings into consideration, we investigated the dynamics of cellular immunity in response to primary and secondary DENV infection in the marmoset model.

Materials and methods

Animals

All animal studies were conducted in accordance with protocols of experimental procedures that were approved by the Animal Welfare and Animal Care Committee of the National Institute of Infectious Diseases, Japan, and the National Institute of Biomedical Innovation, Japan. A total of six male marmosets, weighing 258–512 g, were used. Common marmosets were purchased from Clea Japan Inc.

(Tokyo, Japan) and caged singly at 27 ± 2 °C in 50 ± 10 % humidity with a 12-h light-dark cycle (lighting from 7:00 to 19:00) at Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Tsukuba, Japan. Animals were fed twice a day with a standard marmoset diet (CMS-1M, CLEA Japan) supplemented with fruit, eggs and milk. Water was given ad libitum. The animals were in healthy condition and confirmed to be negative for anti-dengue-virus antibodies before inoculation with dengue virus [24].

Cells

Cell culture was performed as described previously [24]. Vero cells were cultured in minimum essential medium (MEM, Sigma) with 10 % heat-inactivated fetal bovine serum (FBS, GIBCO) and 1 % non-essential amino acid (NEAA, Sigma) at 37 °C in 5 % CO_2. C6/36 cells were cultured in MEM with 10 % FBS and 1 % NEAA at 28 °C in 5 % CO_2.

Virus

DENV type 2 (DENV-2) strain DHF0663 (accession no. AB189122) and strain D2/Hu/Maldives/77/2008NIID (Mal/77/08) were used for inoculation studies. The DENV-2, DHF0663 strain was isolated from a DHF case in Indonesia. The DENV-2 Mal/77/08 strain was isolated from imported DF cases from the Maldives. For all DENV strains, isolated clinical samples were propagated in C6/36 cells and were used within four passages on C6/36 cells. Culture supernatant from infected C6/36 cells was

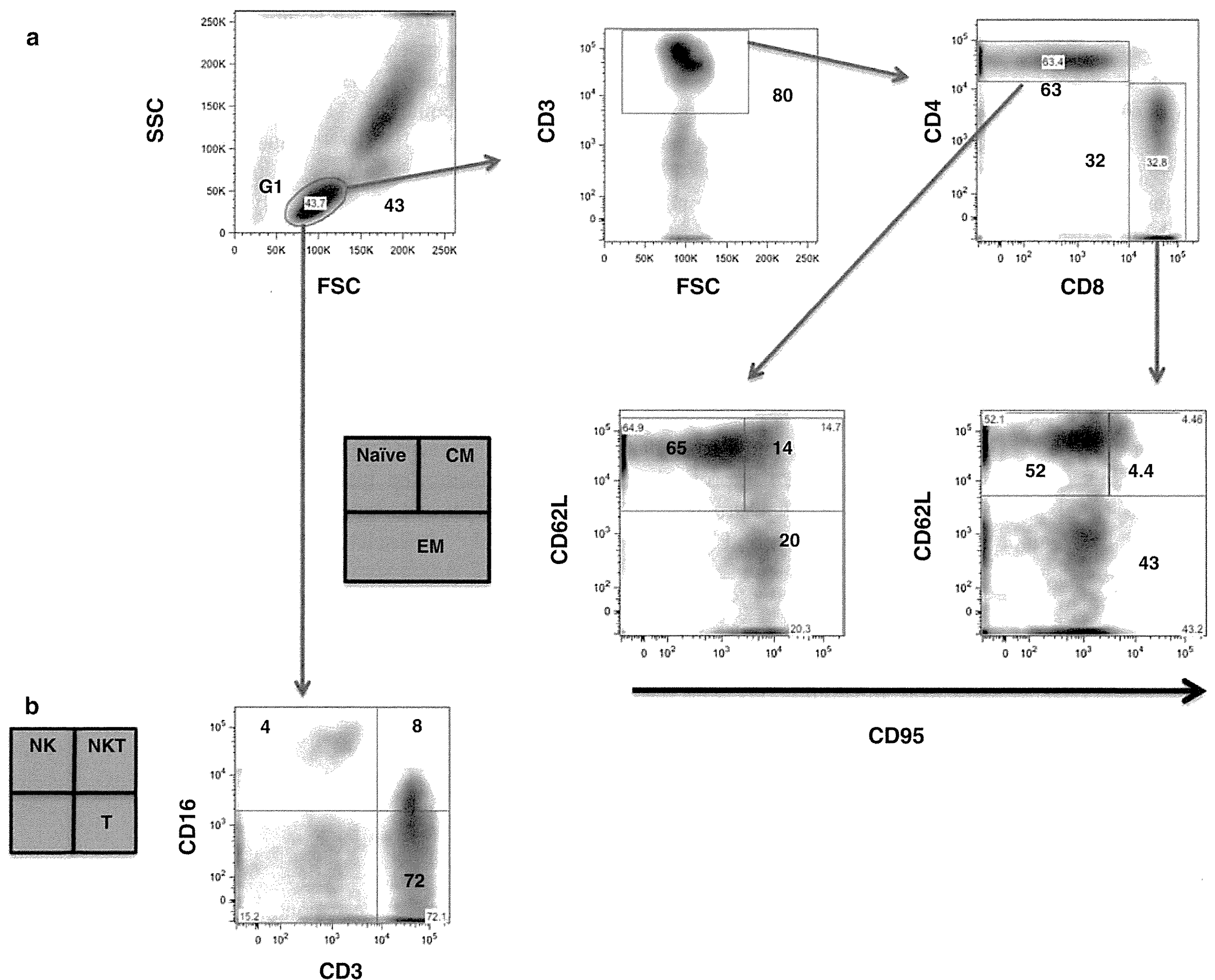


Fig. 1 Flow cytometric analysis of naïve, central/effector memory T cells and NK/NKT cells in marmosets. (**a**) Gating strategy to indentify CD4 and CD8 T, NK and NKT cells. The G1 population was selected and analyzed for CD4 and CD8 T, NK and NKT cells. (**a**) Profiling of naïve, central memory, and effector memory CD4 and CD8 T cells in total CD4 and CD8 T cells. (**b**) Profiling of NK and NKT cells in total lymphocytes. Results shown are representative of three healthy marmosets used in this study

centrifuged at 3,000 rpm for 5 min to remove cell debris and then stored at −80 °C until use.

Infection of the marmosets with DENV

In the challenge experiments, profiling of the key adaptive and innate immune cells in the marmosets after infection with DENV-2 was done. For primary DENV infection, four marmosets were inoculated subcutaneously in the back with either 1.9×10^5 PFU of the DENV-2 Mal/77/08 strain (Cj08-007, Cj07-011) or 1.8×10^4 PFU of the DHF0663 strain (Cj07-006, Cj07-008) [24]. In the case of the DENV re-challenge experiment, two marmosets initially inoculated with 1.8×10^5 PFU of the DHF0663 strain were re-inoculated 33 weeks after the primary challenge with 1.8×10^5 PFU of the same strain (Cj07-007, Cj07-014) [24]. Blood samples were collected on days 0, 1, 3, 7, 14, and 21 after inoculation and were used for virus titration and flow cytometric analysis. Inoculation with DENV and blood drawing were performed under anesthesia with 5 mg/kg of ketamine hydrochloride. Day 0 was defined as the day of virus inoculation. The viral loads in marmosets obtained in a previous study are shown in Supplementary Figure 1 [24].

Flow cytometry

Flow cytometry was performed as described previously [37]. Fifty microliters of whole blood from marmosets was stained with combinations of fluorescence-conjugated monoclonal antibodies; anti-CD3 (SP34-2; Becton Dickinson), anti-CD4 (L200; BD Pharmingen), anti-CD8 (CLB-T8/4H8; Sanquin), anti-CD16 (3G8; BD Pharmingen), anti-CD95 (DX2; BD Pharmingen), and anti-CD62L (145/15; Miltenyi Biotec). Then, erythrocytes were lysed with FACS lysing solution (Becton Dickinson). After washing with a sample buffer containing phosphate-buffered saline (PBS) and 1 % FBS, the labeled cells were resuspended in a fix buffer containing PBS and 1 % formaldehyde. The expression of these markers on the lymphocytes was analyzed using a FACSCanto II flow cytometer (Becton Dickinson). The data analysis was conducted using FlowJo software (Treestar, Inc.). Results are shown as mean ± standard deviation (SD) for the marmosets used in this study.

Table 1 Subpopulation ratios of lymphocytes in marmosets

Subpopulation name	Subpopulation ratios (Mean ± SD: %)
$CD3^+$	75.7 ± 6.4
$CD3^+CD4^+$	65.4 ± 6.8
$CD3^+CD4^+CD62L^+CD95^-$ (CD4 T_N)	65.9 ± 3.7
$CD3^+CD4^+CD62L^+CD95^+$(CD4 T_{CM})	16.4 ± 2.9
$CD3^+CD4^+CD62LCD95^{\pm}$ (CD4 T_{EM})	19.5 ± 2.5
$CD3^+CD8^+$	29.0 ± 8.0
$CD3^+CD8^+CD62L^+CD95^-$ (CD8 T_N)	66.7 ± 10.2
$CD3^+CD8^+CD62L^+CD95^+$(CD8 T_{CM})	4.7 ± 3.6
$CD3^+CD8^+CD62LCD95^{\pm}$ (CD8 T_{EM})	28.8 ± 14.8
CD3CD16 + (NK)	4.2 ± 2.6
$CD3^+CD16^+$ (NKT)	5.1 ± 3.4

SD: Standard deviation

Results shown are mean ± SD from 3 healthy marmosets

Results

Naïve central/effector memory T cells and NK/NKT cells in marmosets

Basic information regarding CD4/CD8 naïve and central/effector memory T cells and NK/NKT cells in common marmosets was unavailable. Thus, we examined the immunophenotypes of lymphocyte subsets in the marmosets (Fig. 1). The gating strategy for profiling the CD4 and CD8 T cells in the marmosets by FACS is shown in Fig. 1a. Human T cells are classically divided into three functional subsets based on their cell-surface expression of CD62L and CD95, i.e., $CD62L^+CD95^-$ naive T cells (T_N), $CD62L^+CD95^+$ central memory T cells (T_{CM}), and $CD62L^-CD95^{\pm}$ effector memory T cells (T_{EM}) [9, 21, 28]. In this study, $CD4^+$ and $CD8^+$ T_N, T_{CM}, and T_{EM} subpopulations were defined as $CD62L^+CD95^-$, $CD62L^+CD95^+$, and $CD62L^-CD95^{\pm}$, respectively (Fig. 1a and Table 1). The average ratio of $CD3^+$ T lymphocytes in the total lymphocytes of three marmosets was found to be 75.7 ± 6.4 %. The average ratio of $CD4^+$ T cells in the $CD3^+$ subset was 65.4 ± 6.8 %. The average ratios of $CD4^+$ T_N, T_{CM}, and T_{EM} cells were 65.9 ± 3.7 %, 16.4 ± 2.9 %, 19.5 ± 2.5 %, respectively. The average ratio of $CD8^+$ T cells in the $CD3^+$ subset was 29.0 ± 8.0 %. The average ratios of $CD8^+$ T_N, T_{CM}, and T_{EM} cells were 66.7 ± 10.2 %, 4.7 ± 3.6 %, 28.8 ± 14.8 %, respectively.

We recently characterized a $CD16^+$ major NK cell subset in tamarins and compared NK activity in tamarins with or without DENV infection [37, 38]. In terms of NKT cells, NK1.1 (CD161) and CD1d are generally used as markers of NKT cells [32]. However, these anti-human NK1.1 and CD1d antibodies are unlikely to cross-react with the NKT cells of the marmosets. Thus, we defined NKT cells as a population expressing both CD3 and CD16 as reported previously [14, 17]. The NK and NKT cell subsets were determined to be $CD3^-CD16^+$ and $CD3^+CD16^+$ lymphocytes in the marmosets. The average ratios of NK and NKT cell subsets in the lymphocytes were 4.2 ± 2.6 % and 5.1 ± 3.4 %, respectively (Table 1). We observed that the proportions of the major lymphocyte

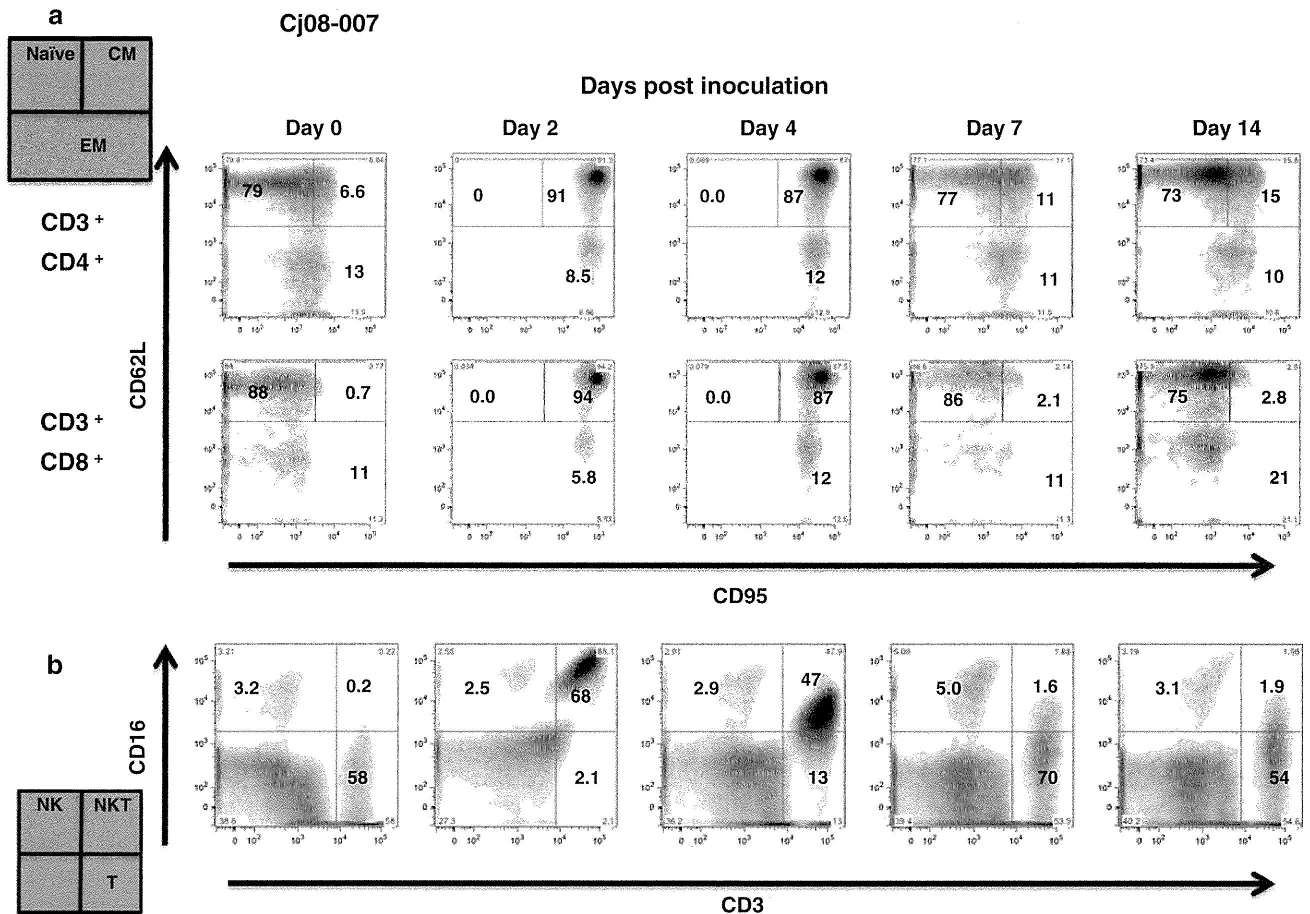


Fig. 2 Profiling of CD4 and CD8 T, NK and NKT cells in marmosets with primary infection with the DENV-2 Mal/77/08 strain. For primary DENV infection, two marmosets were inoculated subcutaneously in the back with 1.9×10^5 PFU of the DENV-2 Mal/77/08 strain. (**a**) Profiling of naïve, central memory, and effector memory CD4 and CD8 T cells in total CD4 and CD8 T cells. (**b**) Profiling of NK and NKT cells in total lymphocytes. (**a-b**) Cj08-007

subsets in the marmosets were similar to those in cynomolgus monkeys and tamarins [37, 38].

Profiling of CD4 and CD8 T, NK and NKT cells in marmosets after primary infection with DENV-2 (Mal/77/08 strain)

We investigated the cellular immune responses against DENV-2 DF strain (Mal/77/08) in marmosets. Dengue vRNA was detected in plasma samples from two marmosets on day 2 postinfection (Supplementary Fig. 1a). For the two marmosets (Cj08-007, Cj07-011), the plasma levels of vRNA reached their peaks at 9.6×10^6 and 7.0×10^6 GE/ml, respectively, on day 4 postinfection. Plasma vRNA was detected in both marmosets on days 2, 4, and 7. We then examined the profiles and frequencies of the CD4 and CD8 T, NK and NKT cells in the infected marmosets (Figs. 2–3 and Table 2). $CD4^+$ T_{CM} cells drastically increased to 88.7 ± 2.8 % from 13 ± 0.4 % between day 0 and day 2 post-inoculation (Table 2). Reciprocally, $CD4^+$ T_N cells decreased to 1.6 ± 3.3 % from 74.1 ± 0.9 % at the same time. $CD4^+$ T_{EM} cells maintained the initial levels throughout the observation period. $CD8^+$ T_{CM} cells increased to 91.9 ± 5.5 % from 2.1 ± 0.8 % between day 0 day 2 post-inoculation, and reciprocally, $CD8^+$ T_N cells decreased to 2.5 ± 4.7 % from 89.9 ± 2.5 % at the same time. In addition, NK cells maintained their initial levels throughout the observation period. However, NKT cells drastically increased to 52.6 ± 17 % from 0.2 ± 0.0 % between day 0 and day 2 post-inoculation. These results suggest that CD4/CD8 T and NKT cells may efficiently respond to the Mal/77/08 strain of DENV.

Profiling of CD4 and CD8 T, NK and NKT cells in the marmosets after primary infection with DENV-2 (DHF0663 strain)

Next, we investigated cellular immune responses against another DENV-2 DHF strain (DHF0663) in marmosets.

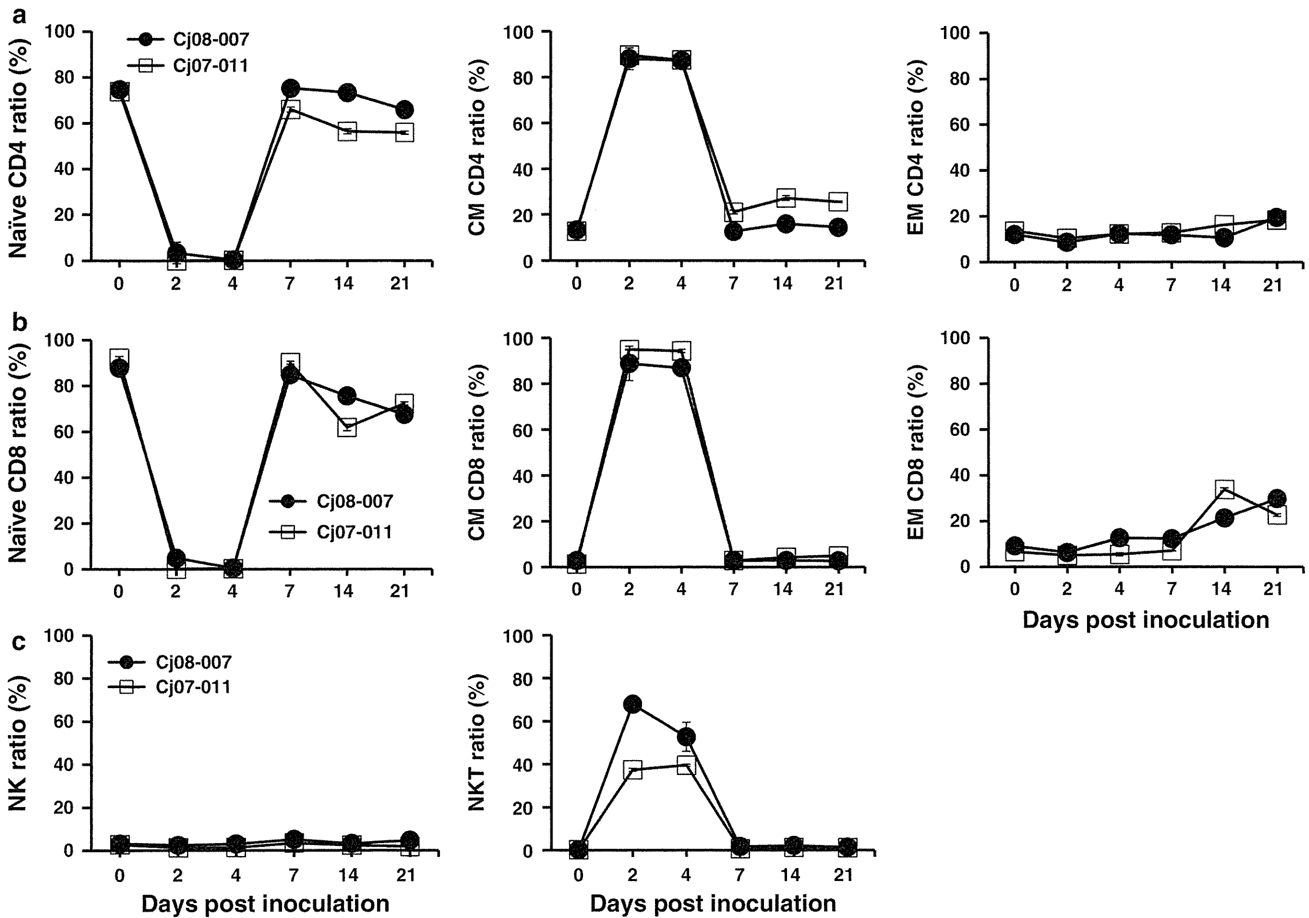


Fig. 3 Frequency of CD4 and CD8 T, NK and NKT cells in marmosets with primary infection with the DENV-2 Mal/77/08 strain. For primary DENV infection, two marmosets were inoculated subcutaneously in the back with 1.9×10^5 PFU of the DENV-2 Mal/77/08 strain. (**a**) Ratios of naïve, central memory, and effector memory CD4 T cells in total CD4 T cells. (**b**) Ratios of naïve, central memory, and effector memory CD8 T cells in total CD8 T cells. (**c**) Ratios of NK and NKT cells in total lymphocytes. (**a-c**) Cj08-007, Cj07-011

Table 2 Subpopulation ratios of lymphocytes in marmosets during primary DENV infection (Mal/77/08)

Subpopulation name		Subpopulation ratio (Mean ± SD: %)					
		Days after inoculation					
		Day 0	Day 2	Day 4	Day 7	Day 14	Day 21
$CD3^+CD4^+CD62L^+CD95''$	(CD4 T_N)	74.1 ± 0.9	1.6 ± 3.3	0.2 ± 0.3	70.5 ± 5.5	64.8 ± 9.7	60.8 ± 5.9
$CD3^+CD4^+CD62L^+CD95^+$	(CD4 T_{CM})	13 ± 0.4	88.7 ± 2.8	87.4 ± 0.2	16.8 ± 5.0	21.6 ± 6.5	20 ± 6.4
$CD3^+CD4^+CD62LCD95^{\pm}$	(CD4 T_{EN})	12.8 ± 0.9	9.5 ± 1.0	12.3 ± 0.4	12.3 ± 0.5	134 ± 3.2	189 ± 1.4
$CD3^+CD8^+CD62L^+CD95^-$	(CD8 T_N)	89.9 ± 2.5	2.5 ± 4.7	0.3 ± 0.3	87.5 ± 3.3	68.7 ± 79	69.8 ± 3.1
$CD3^+CD8^+CD62L^+CD95^+$	(CD8 T_{CM})	2.1 ± 0.8	91.9 ± 5.5	90.6 ± 4.2	2.8 ± 0.5	3.5 ± 08	3.8 ± 1.2
$CD3^+CD8^+CD62LCD95^{\pm}$	(CD8 T_{EN})	7.8 ± 1.6	5.6 ± 0.8	9.0 ± 4.1	9.5 ± 3.1	27.6 ± 72	26.3 ± 4.3
$CD3^-CD16+$	(NK)	2.9 ± 0.2	1.8 ± 0.6	2.2 ± 0.9	4.2 ± 0.9	2.8 ± 04	3.2 ± 1.7
$CD3^+CD16^+$	(NKT)	0.2 ± 0.0	52.6 ± 17	46.1 ± 8.5	1.1 ± 05	1.7 ± 05	1.2 ± 0.2

SD: Standard deviation

Results shown are mean ± SD from two marmosets as shown in Figure 3

Dengue vRNA was detected in plasma samples from the marmosets on day 2 post-infection ([24], Supplementary Fig. 1b). For the two marmosets (Cj07-006, Cj07-008), the plasma vRNA levels were found to be 3.4×10^5 and 3.8×10^5 GE/ml on day 2 and 2.0×10^6 and 9.4×10^5 GE/ml, respectively, at the peak on day 4 post-infection and became undetectable by day 14. Thus, we examined the profiles and frequencies of the $CD4^+$ and $CD8^+$ T, NK and NKT cells in these DENV-infected marmosets (Fig. 4–5 and Table 3). It was found that on day 7 post-inoculation, $CD4^+$ and $CD8^+$ T_N cells decreased, and in contrast, the T_{CM} populations increased in both marmosets; however, the changes in proportion were much less pronounced than in the case of the marmosets infected with the DF strain. We observed no consistent tendency in the kinetics of $CD4^+$ and $CD8^+$ T_{EM} cells nor in NK and NKT cells. These results suggest that the strength of T cell responses may be dependent on the strain of DENV.

Profiling of CD4 and CD8 T, NK and NKT cells in marmosets re-challenged with a DENV-2 strain

In order to examine the cellular immune responses against re-challenge with a DENV-2 DHF strain in the marmoset model, marmosets were infected twice with the same DENV-2 strain (DHF0663) with an interval of 33 weeks after the primary infection. The results showed that vRNA and NS1 antigens were not detected in plasma and that the neutralizing antibody titer was obviously increased after the secondary infection. The data indicated that the primary infection induced protective immunity, including a neutralizing antibody response to re-challenge with the same DENV strain ([24]; Supplementary Fig. 1c). We also investigated the profiles of the CD4 and CD8 T, NK and NKT cells in the marmosets (Cj07-007, Cj07-014) that were re-challenged with the same DENV-2 strain (DHF0663) (Figs. 6–7). $CD4^+$ T_{CM} cells drastically increased on day 14 post-inoculation. On the other hand,

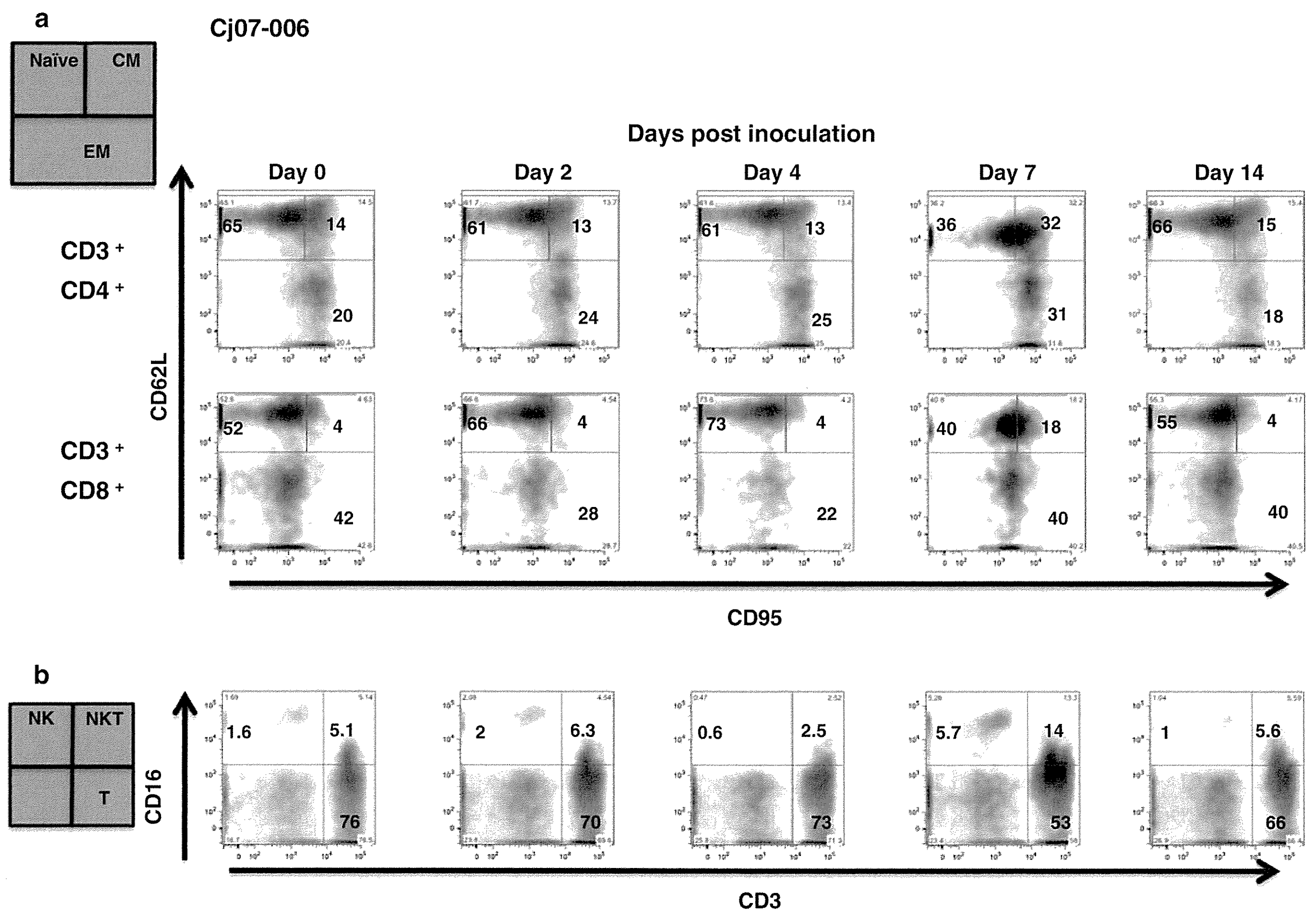


Fig. 4 Profiling of CD4 and CD8 T, NK and NKT cells in marmosets with primary infection with the DENV-2 DHF0663 strain. For primary DENV infection, two marmosets were inoculated subcutaneously in the back with 1.8×10^4 PFU of the DENV-2 DHF0663 strain. (**a**) Profiling of naïve, central memory, and effector memory CD4 and CD8 T cells in total CD4 and CD8 T cells. (**b**) Profiling of NK and NKT cells in total lymphocytes. (**a-b**) Cj07-006

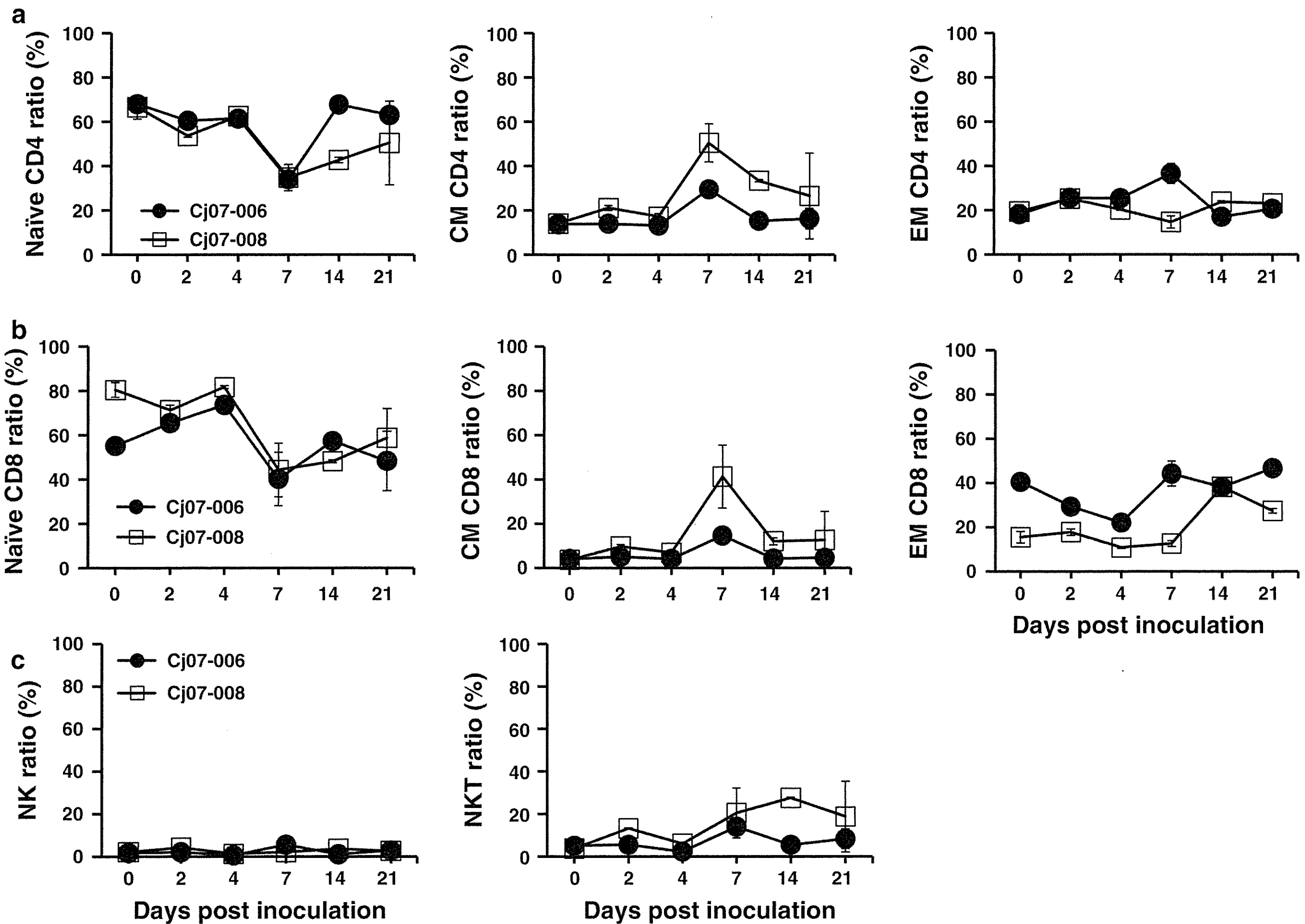


Fig. 5 Frequency of CD4 and CD8 T, NK and NKT cells in marmosets with primary infection with the DENV-2 DHF0663 strain. For primary DENV infection, two marmosets were inoculated subcutaneously in the back with 1.8 x 10^4 PFU of the DENV-2 DHF0663 strain. (**a**) Ratios of naïve, central memory, and effector memory CD4 T cells in total CD4 T cells. (**b**) Ratios of naïve, central memory, and effector memory CD8 T cells in total CD8 T cells. (**c**) Ratios of NK and NKT cells in total lymphocytes. (**a-c**) Cj07-006, Cj07-008

Table 3 Subpopulation ratios of lymphocytes in marmosets during primary DENV infection (DHF0663)

Subpopulation name		Subpopulation ratios (Mean ± SD: %)					
		Days after inoculation					
		Day 0	Day 2	Day 4	Day 7	Dayl 4	Day 21
$CD3^+CD4^+CD62L^+CD95^-$	(CD4 T_N)	67.3 ± 3.6	57.0 ± 4.0	61.9 ± 0.9	34.4 ± 3.6	55.2 ± 14	56.7 ± 13
$CD3^+CD4^+CD62L^+CD95^+$	(CD4T_{CM})	13.9 ± 1.3	17.5 ± 4.1	15.2 ± 2.5	40.0 ± 13	33.8 ± 10	21.3 ± 12
$CD3^+CD8^+CD62L^-CD95^\pm$	(CD4 T_{EM})	18.8 ± 2.2	25.3 ± 0.9	22.8 ± 2.9	25.6 ± 13	20.3 ± 4.0	21.8 ± 1.5
$CD3^+CD8^+CD62L^+CD95^-$	(CDS T_N)	67.8 ± 14	68.4 ± 3.7	77.7 ± 4.6	42.2 ± 7.4	52.7 ± 5.5	53.5 ± 9.8
$CD3^+CD8^+CD62L^+CD95^-$	(CDS T_{CM})	3.9 ± 0.6	7.4 ± 2.8	5.5 ± 1.6	28 ± 17	8.1 ± 4.6	8.6 ± 8.9
$CD3^+CD8^+CD62L^-CD95^\pm$	(CDS T_{EM})	28 ± 14	23.5 ± 6.7	16.4 ± 6.5	28.3 ± 18	38.2 ± 1.9	37.0 ± 11
$CD3^-CD16^+$	(NK)	4.7 ± 1.0	4.2 ± 1.9	2.0 ± 1.1	6.3 ± 2.3	5.1 ± 2.2	7.3 ± 1.2
$CD3^+CD16^+$	(NKT)	7.8 ± 1.0	9.3 ± 4.5	5.9 ± 2.6	22.6 ± 8.4	20.6 ± 10	17.3 ± 10

SD: Standard deviation

Results shown are mean ± SD from 2 marmosets as shown in Figure 5