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IL-28A Inhibits IgM, IgG, and IgA Antibody Production Induced by Anti-CD40 in Combination with Cytokine IL-2 and IL-10

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Abstract

Interleukin (IL)-28A is an immuno-regulatory cytokine that exerts effects similar to interferon (IFN)-α/β including anti-viral, anti-proliferative, and anti-tumor activity. We examined whether IL-28A affects antibody production of human B cells induced by anti-CD40 monoclonal antibody (mAb) immobilized on L cells expressing FcγRII (CDw32) (L/CDw32) in the presence of IL-10 and IL-2. IL-28 reduced IgM, IgG, and IgA antibody production induced by anti-CD40 mAb plus IL-2 and IL-10 in a dose-dependent manner. Kinetic analysis showed that IL-28A suppressed the IgM, IgG, and IgA antibody responses throughout the entire culture periods, suggesting that the inhibition is not due to a shift in the kinetics of antibody production. IL-28A exerted its suppressive activity when added within 3 days after initiation of a 10-day culture. The IL-28A-mediated inhibition of IgG and IgA antibody production was partly accounted for by a class switch recombination (CSR), because the expression of activation-induced cytidine deaminase (AID) by anti-CD40 mAb plus IL-2/IL-10 was reduced by the addition of IL-28A. Together, IL-28A prevents IgG and IgA antibody responses, at least through impairment of AID mRNA synthesis in human peripheral B cells.

Introduction

Interleukin (IL)-28A, IL-28B, and IL-29 (also known as IFN-λ2, IFN-λ3, and IFN-λ1) are a new family of cytokines that behave similarly to type I interferon (IFN)[1)2)]. IL-28 and IL-29 have anti-viral activity, anti-tumor actions, and also immuno-regulatory functions[3-5)]. For example, IL-28A protected HepG2 cells from virus-induced cytopathic effects[2)], and IL-28-secreting fibrosarcomas reduced tumor growth through activation of neutrophils, NK cells, and CD8 T cells[3)]. IL-28 also increased total IgG antibody production induced by anti-CD40 mAb plus IL-4 in human B cells, while it reduced IgA production induced by anti-CD40 mAb together with TGF-β[6)].

During T cell-dependent antibody responses, B cells undergo activation, proliferation, and differentiation on receipt of helper T cell signals, including CD40-ligand (CD40-L) and cytokines[7)8)]. CD40-L is transiently expressed on activated T cells, and the interaction of CD40-L with CD40 on B cells in the presence of appropriate cytokines induces an immunoglobulin class switch recombination (CSR), resulting in generation of IgG-, IgA-, or IgE-producing cells[9)]. We and others have demonstrated that human B cells effectively undergo production of IgM, IgG, and IgA antibody following stimulation with anti-CD40 mAb immobilized on L cells expressing FcγRII (CDw32) (L/CDw32) in combination with IL-2 and IL-10[10)11)], which is further enhanced by ligation of CD27 on B cells[11)]. CSR events contribute to the change of effector function of an antibody, which is initiated by activation-induced cytidine deaminase (AID) through deaminating cytidine[12)13)]. Antibody diversity is increased by CSR

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and somatic hypermutation, which may play a crucial role in the protection against a wide variety of viral infections[14].

In the present study, we examined whether IL-28A affects the antibody production induced by anti-CD40 mAb immobilized on L/CDw32 plus IL-2/IL-10 in human B cells. IL-28A reduces the induction of IgM, IgG, and IgA antibodies induced by the anti-CD40 plus cytokines (IL-2/IL-10). These findings would be useful for the understanding of IL-28-mediated modulation of immune responses.

Materials and methods

Isolation of human peripheral B cells

B cells were purified from peripheral blood lymphocytes using magnetic beads, as previously described[11]. Briefly, the buffy coat layer was collected from heparinized peripheral blood samples. The cells were diluted with phosphate-buffered saline (PBS), layered on Ficoll-Isopaque separation medium, and centrifuged. B cells were positively selected from the isolated monolayer cell suspension by rosetting with magnetic Dybabeads coated with anti-CD19 mAb (Dynal Biotech, Oslo, Norway). The separation was carried out according to the manufacturer's protocol. More than 95% of the CD19-positive B cell populations were IgM-positive (Yonekubo et al., unpublished observation).

Cell cultures

Cultures were carried out in RPMI-1640 medium containing 10% (v/v) fetal bovine serum (FBS), 50 μM 2-mercaptoethanol, 2 mM L-glutamine and 100 μg/ml kanamycin. B cells were cultured as previously described[11]. Briefly, B cells (2×10^4/well) were cultured with anti-CD40 mAb plus recombinant IL-2 and IL-10 (PeproTech, London, UK) in the presence or absence of recombinant IL-28A (IL-28A) (R & D Systems, Minneapolis, MN, USA) for various numbers of days. Anti-CD40 mAb was purified by ammonium sulfate precipitation of ascites fluid injected intraperitoneally with a hybridoma cell line (G28-5 ; American Type Culture Collection ; Manassas, VA, USA). In some experiments, IL-28A was affinity-purified from culture supernatants of 293 cells transfected with a p3xFlag-CMV-9 expression vector (Sigma, St. Louis, MO, USA) containing IL-28 cDNA (Hata et al., unpublished observation). A more detailed explanation for the expression vector will be provided upon request. L/CDw32 cells, which were kindly provided by Dr. K. Moore (DNAX, Palo Alto, CA, USA)[15], were irradiated (9000 rad) and stored at −800°C until use.

Quantitation of Ig by enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was performed as previously described[11]. Briefly, ELISA plates were coated with anti-human IgM, anti-human IgG, or anti-human IgA, followed by incubation overnight. Samples were added to plates and incubated for 1 h. After several washes, alkaline phosphatase-labeled goat IgG specific for human isotypes (ICN Pharmaceuticals Inc., Aurora, OH, USA) was added. After 1 h incubation, color was detected by addition of p-nitrophenyl phosphate for 30 min.

Separation of CD27-positive B cells by autoMACS

B cells were further subdivided into CD27-positive ($CD27^+$) and CD27-negative ($CD27^-$) cells by an autoMACS cell sorter (Miltenyi Biotec, Bergisch Gladbach, Germany), as described previously[11]. Briefly, B cells were incubated with anti-CD27 mAb MACSbeads (Miltenyi Biotec). Following several washes, the cells were applied to a MACS column (Miltenyi Biotec) to separate $CD27^+$ from $CD27^-$ cells.

Reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) was carried out, as previously described[16]. Briefly, total RNA isolated from human B cells was reverse-transcribed using an RNA PCR kit (Takara Bio Inc., Otsu, Japan). PCR reactions were carried out with Taq polymerase (Takara Bio Inc.) using the following primers : human AID (forward, 5′-ATGGACAGCCTCTTGATGAAC-3′ ; reverse, 5′-TCAAAGTCCCAAAGTACGAAATG-3′) and human GAPDH (forward, 5′-TGGAAGGGCTCATGACCAC-3′ ; reverse, 5′-CCATGTAGGCCATGAGGTC-3′), generating fragments of 597 bp and 1,008 bp, respectively. PCR products were resolved on 1.5% agarose gels and visualized with ethidium bromide.

Statistical analysis

Data are expressed as mean±SD for each group. Statistical analysis was carried out by one-factor ANOVA with the SigmaStat for Windows software package. Statistical significance was set at 0.05.

Results

IL-28A prevents induction of IgM, IgG, and IgA antibody production by anti-CD40 mAb plus IL-2 and IL-10 in human B cells

Human B cells (2×10^4/well) were co-cultured with anti-CD40 mAb presented on the irradiated L/CDw32 cells with IL-2 and IL-10 in the presence or absence of rIL-28A for 7 days, and culture supernatants were assayed by ELISA. Anti-CD40 mAb synergized with both IL-2 and IL-10 to induce IgM, IgG, and IgA antibody responses (Fig. 1), as reported[10][11]. The addition of rIL-28A to the culture reduced the IgM, IgG, and IgA antibody production in a dose-dependent manner, with marked inhibition at 10 ng/ml. Our preparation

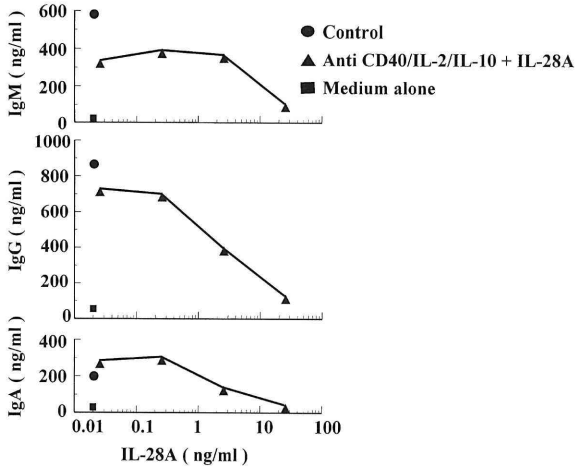

Fig. 1 IL-28A reduces IgM, IgG, and IgA antibody production induced by anti-CD40 mAb together with IL-2 and IL-10. Human peripheral B cells (2×10^4/well) were cultured for 7 days with anti-CD40 mAb plus IL-2/IL-10 in the presence or absence of IL-28A. Culture supernatants were harvested, and the levels of IgM, IgG, and IgA in the supernatants were measured by ELISA. Results are expressed as means±SD of triplicate cultures. The results are representative of three experiments.

of IL-28A also showed a pattern of inhibition similar to that of rIL-28A (Yonekubo et al., unpublished observation). Thus, rIL-28A (IL-28A) was employed in this study.

CD27 is considered to represent a useful marker for memory B cells[17)18)]. We previously demonstrated that stimulation with anti-CD40 mAb plus IL-2/IL-10 induces efficient antibody production of $CD27^+$ B cells[11)]. The antibody responses of enriched $CD27^+$ cells induced by anti-CD40 mAb plus IL-2/IL-10 were also reduced by IL-28A (Yonekubo et al. unpublished observation). These results suggest that IL-28A inhibits the antibody production induced by anti-CD40 mAb plus IL-2/IL-10.

IL-28A does not impair ^{3}H-Tdr incorporation induced by anti-CD40 mAb plus IL-2/IL-10

To examine whether IL-28A affects proliferative responses of human B cells, B cells were stimulated with anti-CD40 mAb plus IL-2/IL-10 together with rIL-28A. Following culture for 7 days, the cultures were pulsed with ^{3}H-TdR for the final 6 h and harvested. The incorporation of ^{3}H-TdR was unaffected by the addition of rIL-28A, compared with controls (Fig. 2), suggesting that IL-28A does not affect the proliferative responses of B cells induced by anti-CD40 mAb plus IL-2/IL-10.

Kinetics of IL-28A-mediated inhibition of IgM, IgG, and IgA antibody production by anti-CD40 mAb plus IL-2/IL-10 in human B cells

To examine whether IL-28A affects the kinetics of antibody responses induced by anti-CD40 plus IL-2/

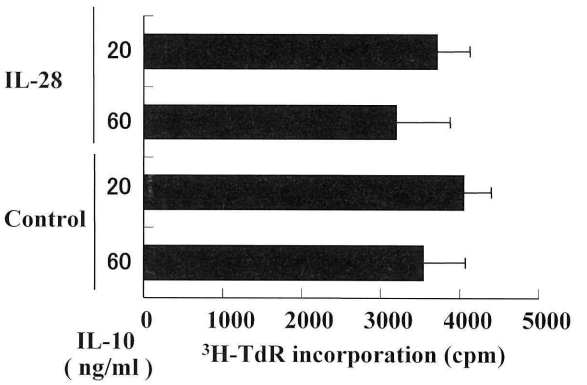

Fig. 2 IL-28A does not affect proliferative responses induced by stimulation with anti-CD40 mAb plus IL-2/IL-10. B cells were cultured with anti-CD40 mAb plus IL-2/IL-10 in the presence or absence of IL-28A. Following stimulation for 7 days, the cells were pulsed with ^{3}H-Tdr for the final 6 h. Results are expressed as means±SD of triplicate cultures. Experiments were done three times, with essentially similar results.

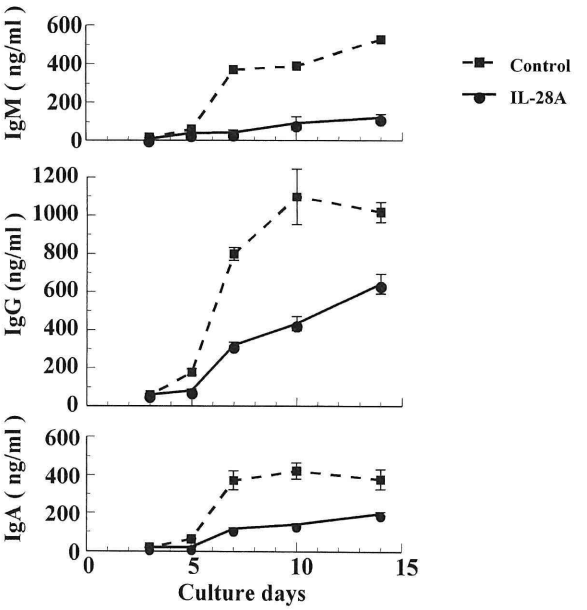

Fig. 3 Kinetics of IL-28A-medicated prevention of IgM, IgG, and IgA antibody responses induced by stimulation with anti-CD40 mAb together with IL-2 and IL-10. B cells were cultured with the stimulants for the indicated time periods, as described in Fig. 1. The supernatants were assayed by ELISA. Results are expressed as means±SD of triplicate cultures. The results are representative of three experiments.

IL-10, B cells were cultured with anti-CD40 mAb immobilized on irradiated L/CDw32 cells with IL-2/IL-10 in the presence or absence of IL-28A for the indicated numbers of days. As shown in Fig. 3, IL-28A inhibited the IgM, IgG, and IgA antibody responses throughout the culture periods. These results suggest that IL-28A

reduces antibody production induced by anti-CD40 plus IL-2/IL-10.

IL-28A exerts suppressive activity against the early, but not late phase of antibody responses induced by anti-CD40 mAb plus IL-2/IL-10

To address which phases of the antibody responses induced by anti-CD40 mAb plus IL-2/IL-10 are affected by IL-28A, IL-28A was added on the first day or various numbers of days after initiation of the culture. Following the initiation of culture, the culture supernatants were harvested and assessed by ELISA. The simultaneous addition of IL-28A substantially prevented the antibody responses (Fig. 4). The suppressive activity of IL-28A became less effective when added on day 3 of a 7-day incubation, with almost no inhibition when it was added during days 5-7. These results suggest that IL-28A must be added during an early phase of the antibody responses to exert its suppressive activity.

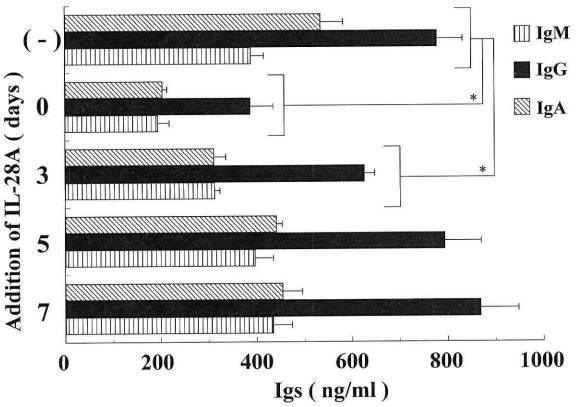

Fig. 4 IL-28A affects antibody responses in early, but not late phase of stimulation with anti-CD40 mAb plus IL-2/IL-10. IL-28A was added on the indicated day to cultures containing anti-CD40 mAb plus IL-2/IL-10. At day 7 after stimulation, supernatants were harvested and assessed by ELISA. Results are expressed as means±SD of triplicate cultures. The results are representative of three experiments. *Significantly different from control culture without IL-28A. $p<0.05$

IL-28A impairs AID expression levels induced by anti-CD40 mAb plus IL-2/IL-10

Recent studies have clearly demonstrated the absolute requirement of AID for CSR and somatic hypermutation[12]. To examine whether IL-28A affects AID expression, B cells were stimulated with anti-CD40 mAb plus IL-2/IL-10 in the presence or absence of IL-28A for various time periods, and the levels of AID mRNA expression were determined by RT-PCR. The AID expression induced by anti-CD40 mAb plus IL-2/IL-10 reached its peak around days 3–5 after stimulation, followed by a decline (Fig. 5). The addition of IL-28A resulted in a substantial decrease in AID expression levels throughout the culture period, suggesting that IL-28A affects AID synthesis by anti-CD40 mAb plus IL-2/IL-10 in human B cells.

IL-28A partially reduces antibody responses induced by anti-CD40 mAb plus IL-2/IL-4

Because IL-28A has the IL-10R2 chain in common with IL-10, we examined whether IL-28A modulates antibody responses following stimulation with anti-CD40 mAb plus IL-2/IL-4, which do not include common IL-10R2[19)20)]. Although the magnitude of the antibody responses induced by anti-CD40 mAb plus IL-2/IL-4 was less pronounced than those induced by anti-CD40 mAb plus IL-2/IL-10, IL-28A partially inhibited IgM, IgG, and IgA antibody responses to the stimulation (Fig. 6). Thus, IL-28A impairs antibody responses induced by cytokines independent of the class II cytokine family.

Discussion

The family of type I IFN-like cytokines IL-28A, IL-28B, and IL-29 have anti-viral and anti-tumor activities similar to IFN-α/β[1-3)5)]. In addition, IFN-α possesses immuno-modulatory functions[21)]. For example, IFN-α modulates antibody responses, depending on the dose and time of addition during culture[22)23)]. We

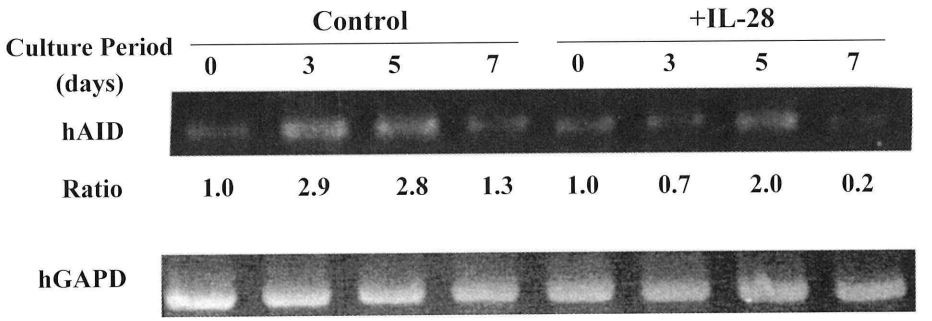

Fig. 5 IL-28A reduces AID expression levels induced by stimulation with anti-CD40 mAb and IL-2/IL-10. B cells stimulated with anti-CD40 mAb plus IL-2/IL-10 in the presence or absence of IL-28A for various time periods were assessed by AID mRNA expression using PCR. Levels of AID mRNA expression were normalized to those of GAPDH and were expressed as a multiple of the AID levels from unstimulated cells. Experiments were done four times, with essentially similar results. *Significantly different from control medium alone.

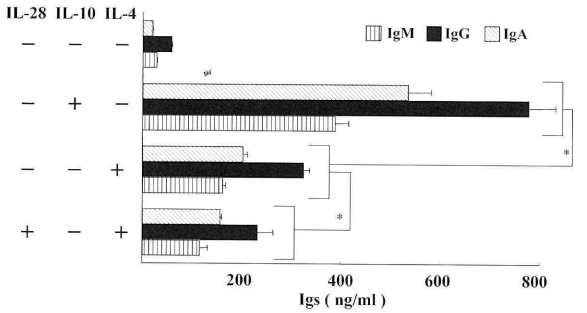

Fig. 6 IL-28A partially reduces antibody responses induced by stimulation with anti-CD40 mAb plus IL-2/IL-10. B cells were cultured with anti-CD40 mAb plus IL-2/IL-4 for 7 days, and the antibody responses in the culture supernatants were assayed using ELISA. Experiments were done three times, with essentially similar results. *Significantly different from control culture without IL-28A. $p<0.01$

and others have demonstrated that anti-CD40 mAb on CDw32 cells in the presence of IL-2 plus IL-10 stimulates human peripheral blood B cells to produce antibodies[11]. In the present study, we investigated whether IL-28 modulates the antibody responses of human B cells in response to cytokine stimulation.

Treatment with IL-28A reduced IgM, IgG, and IgA antibody responses induced by anti-CD40 mAb together with IL-2 plus IL-10 (Fig. 1). Consistent with our findings, IL-28/IL-29 partially suppressed IgA antibody responses induced by anti-CD40 mAb plus TGF-β[6]. IL-28 did not shift the kinetics of the antibody responses induced by anti-CD40 mAb plus Il-2/IL-10 (Fig. 3), suggesting that affecting the kinetics of the response did not account for IL-28A-induced inhibition of antibody responses. Moreover, addition of IL-28A was necessary during the first 3 days of a 13-day culture for efficient prevention of antibody responses, suggesting that IL-28A functions during the early, but not late, phases of antibody responses, such as antibody secretion into the culture medium.

Certain levels of AID expression are required for CSR and somatic hypermutation of B cells[12)24)]. Ligation of CD40 with anti-CD40 mAb upregulated AID expression of IL-2/IL-10-stimulated B cells, which peaked 3–5 days after stimulation (Fig. 5). However, the CD40 mAb-induced AID upregulation was substantially prevented by co-incubation with IL-28A, suggesting that IL-28A somehow modulates CSR induced by anti-CD40 mAb plus IL-2/IL-10. It should be mentiond, however, that differentiation of IgM-committed B cells might also be blocked by IL-28A.

B cell differentiation induced by T cell-derived signals (CD40-L and/or cytokines) accompanies cell proliferation[7)8)]. However, the anti-CD40-induced proliferative responses of B cells in the presence of IL-2/IL-10 was unaffected by IL-28A (Fig. 2), as assessed by ^{3}H-TdR uptake. These results might suggest a different sensitivity to IL-28A between cytokine-induced B cell differentiation and proliferation : a strong and/or sustained signal may be required for inhibition of proliferation compared with differentiation.

IL-28A/IL-29B/IL-29 are new members of the class II family of cytokine receptors (CRF) that includes IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, and interferons[19)20)]. Although the ligand-binding chain for IL-10, IL-10R1, is distinct from that of IL-28A/Il-28B, they share a common second chain, IL-10R2, to form their active receptor complexes[19)20)25)]. It is proposed that binding of IL-10 to IL-10R1 induces a conformational change of IL-10R2 that enables association of the newly formed ligand-receptor complexes[26)], suggesting that IL-10 shares some functions with IL-28/IL-29. Similar to IL-10, IL-28A/IL-28B enhanced total IgG responses induced by anti-CD40 mAb plus IL-4[6)]. However, IL-28/IL-29 prevented antibody response induction by anti-CD40 mAb alone or anti-CD40 plus IL-2/IL-10[6)11)] (Fig. 1 and 3). Thus, the actions of IL-28/IL-29 may depend on the cytokine microenvironment.

Although IL-28Rs are composed of receptors distinct from type I IFN-Rs, IL-28R-mediated signaling seems to be similar to those of IFN-α-Rs[5)20)27)28)], thus functionally resembling IFN-α in the display of anti-tumor activity[3)], antiviral action[1)2)], and immunomodulation[4)6)] (Fig. 1). IFN-α has been employed clinically for treatment of certain diseases including chronic lymphatic leukemia, malignant melanoma, and chronic hepatitis B/C[21)29–31)]. However, the clinical use of IFN-α is limited by inherent and/or acquired resistance to this treatment and adverse effects such as fever and myelosuppression[29–31)]. Further elucidation of IL-28/IL-29-mediated action may hopefully result in substitution of IL-28/IL-29 for IFN-α in the management of immune-mediated diseases and viral infection, because the expression profile of IL-28/IL-29Rs is rather limited compared with that of type I IFNARs[20)27)].

In summary, in the present study we clarified that IL-28A reduces antibody production in response to anti-CD40mAb plus IL-2/IL-10, at least by modulating AID expression in murine B cells. These findings would provide useful information for understanding IL-28-induced immunomodulation.

Acknowledgments

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IL-28A は抗 CD40 抗体、IL-2、IL-10 によって誘導される IgM、IgG、IgA 抗体産生を抑制する

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インターロイキン(IL)-28A は近年クローニングされたサイトカインであり、抗ウィルス作用、細胞増殖抑制作用、抗腫瘍作用などインターフェロン(IFN)-α/β と類似の作用を持っていることが報告されている。今回、我々は Fcγ 受容体(FcγR)II 陽性繊維芽細胞をマイクロプレートに接着させ、抗 CD40 抗体、IL-10、IL-2 でヒト末梢血 B 細胞を刺激することによって得られる IgM、IgG、IgA 抗体産生に IL-28A がいかなる影響を与えるかを検討した。IL-28A はこれらの刺激によって誘導される IgM、IgG、IgA 抗体産生を容量依存的に抑制した。抗体産生の時間経過を検討したが、細胞培養の全期間にわたって IL-28A による抑制が観察されたことから、この抑制は抗体産生動態の変化によるものではないということが示された。IL-28A による抑制効果は 10 日間の細胞培養という条件下では、最初の 3 日間に添加されることが必要であった。CD40 抗体、IL-10、IL-2 刺激によって activation-induced cytidine deaminase (AID)mRNA の発現が亢進したが、この発現も IL-28A によって抑制されることから、IL-28A は B 細胞のクラススイッチを抑制していると推測される。以上より、IL-28A は少なくとも AID mRNA 合成の抑制を介して、CD40 抗体、IL-10、IL-2 刺激によってもたらされるヒト B 細胞の抗体産生を抑制していることが明らかとなった。

〈キーワード〉 ヒト IL-28、B 細胞、CD40、抗体産生