NOTE  

Immunology  

Cell Surface Expression of a Chimeric Protein Containing Mouse Immunoglobulin G1 Fc Domain and its Immunological Property  

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ABSTRACT. Recently we reported that a chimeric molecule containing mouse transferrin receptor and immunoglobulin G1 (IgG1) Fc, mTR-Fc, induced higher immune responses and can be used as a vaccine adjuvant. In this study, the immunological property of the molecule was investigated. Although, the mTR-Fc did not activate complement classical pathway, it was recognized by activated macrophage as like intact IgG Fc, which is recognized by macrophage via Fcγ receptor. In addition, we found that splenocyte simultaneously exposed to lipopolysaccharide (LPS) and mTR-Fc produced higher amount of interleukin-10, comparing to that exposed to only LPS. These results suggest that the mTR-Fc molecules conserved the IgG Fc property to biasing immune responses via modulation of cytokine production by antigen presenting cell.  

KEY WORDS: Fc, IgG1, IL-10.  

Receptors for immunoglobulins Fc region (FcR) on antigen presenting cells (APCs) play an important role in the activation of immune reactions against infectious organisms [3, 6, 9, 10, 13, 17]. It was known that immunization with a complex of antigens and antibodies induced much greater level of immune responses comparing to immunizations with only antigens [18]. This is due to an increased uptake of antigen-antibody complex into APC through the FcRs on the surface of APC, resulting in increased processing and presentation of antigens [18]. To apply the phenomenon for the improvement of vaccines for virus diseases, we constructed a chimeric protein which contains a membrane domain of mouse transferrin receptor and a Fc domain of immunoglobulin (IgG1), mTR-Fc, and reported that the immunization with virus particles harboring the mTR-Fc molecules on its surface induced much greater level of immune responses than that with normal virus particles [16]. However, the mechanism of the stronger immune responses by the mTR-Fc and the properties of the mTR-Fc molecules have not been known so well. In this study, using some cell lines stably expressing the mTR-Fc molecule as a membrane protein on its surface (Fig. 1, inserted small panel), the immunological properties of the mTR-Fc molecule was further investigated.  

The mTR-Fc expressing cell lines had been established as previously reported [16]. Briefly, rabbit kidney derived RK13 cells were transformed with the plasmids coding mTR-Fc molecule and G418 resistant gene and then G418 resistant cell clones were selected. The expression level of mTR-Fc on the cell surface was measured by ELISA as described previously [16]. Among the obtained G418 resistant clones, expression level of mTR-Fc was varied (Fig. 1). To examine whether mTR-Fc molecule can activate complement classical pathway via its Fc derived domain, these established G418 resistant cells were treated with rabbit serum as a source of complement and then the mortality of the treated cells were measured as described previously [8]. It had been confirmed that, in this condition, normal RK13 cells were not lysed but when RK13 cells were beforehand opsonized with anti-RK13 cell antibodies (α-RK13 Abs) the cells were lysed by the rabbit complement activated through classical pathway (data not shown). In this experiment, RK/GFP cells expressing enhanced green fluorescent protein instead of mTR-Fc [16], which had been constructed by the same manner to mTR-Fc expressing cell lines, was used as a control. As shown in Fig. 1, RK/GFP cells showed high mortality only when the RK/GFP cells beforehand opsonized with α-RK13 Abs. In spite of the existence of molecules containing Fc domain on the cell surface, any established G418 resistant cell clone did not show high mortality (Fig. 1). In addition, positive correlation between the mortality and the mTR-Fc expression level was not observed (correlation coefficient = – 0.32). These results indicate that mTR-Fc molecule does not activate complement classical pathway. As shown in Fig. 1, three clones were designated as RK/SK (null), RK/SK(low) and RK/SK (high), which showed no-, low and high level of mTR-Fc expression, and used for the further investigations.  

The activated complement fragments stimulate innate and adaptive immune responses [11]. To investigate whether the mTR-Fc molecule can enhance innate and/or adaptive immune responses independent of complement classical pathway, further experiments were carried out. To confirm whether Fc derived domain in the mTR-Fc mole-
The molecule can induce antibody-dependent cell-mediated cytotoxicity (ADCC), RK13 cells and RK/SK cells were co-cultured with activated mouse peritoneal macrophage and the lysis of target RK13 and RK/SK (high) cells were measured. Peritoneal macrophages were harvested from female BALB/c mice and cultured for 16 hr in the supernatant of mouse splenocyte culture, which had been stimulated with 10 µg/ml concanavalin A, as a source of macrophage activating cytokines [12]. And then 2 × 10^6 cells/well the activated macrophages were added into a confluent cell culture of RK13 or RK/SK (high) in 96 well cell culture plate. After the co-cultivation for 20 hr, the lysis of RK13 or RK/SK (high) cells were detected as lactate dehydrogenase (LDH) release, using commercial cytotoxicity assay system (Cyto96 Non-radio active cytotoxicity Assay, Promega, WI, U.S.A.) according to the manufacture’s instruction. RK13 cells without any opsonization were not lysed in this condition (Fig. 2A). It had been confirmed that RK13 cells beforehand opsonized with α-RK13 Abs were lysed (data not shown). The statistical difference of LDH release from RK13 and RK/SK cells was determined by two-sided t-test. As shown in Fig. 2A, there was more significantly lysis of RK/SK(high) cells by activated macrophage without any further opsonization than lysis of RK13 cells (p<0.01). The results strongly suggested that mTR-Fc molecules on the surface of RK/SK (high) cells were recognized by FcgR on the activated macrophage and then RK/SK cells were lysed by ADCC.

The results that mTR-Fc molecule can be recognized by FcγR on APC prompted us to investigate whether mTR-Fc molecule can induce higher level of adaptive immune responses. In order to settle with the issue, female BALB/c mice were administered with 1 × 10^6 RK13 or RK/SK (high) cells into footpad. Two weeks after the administration, sera samples were harvested and IgG reacting with RK13 cells in the sera samples were detected by ELISA as described previously [8]. The statistical difference was determined by two-sided t-test. As shown in Fig. 2B, the immunization with RK/SK (high) cells induced significant higher level of anti-RK13 IgG production than that with parental RK13 cells (p<0.05). Higher level productions of IgG1 and IgG2a reacting RK13 were also observed (data not shown). The result indicates that mTR-Fc induced higher level of adaptive immune responses. It was reported that complex of antigens and antibodies induced much greater level of immune responses because of an increased uptake of antigen-antibody complex into APC, processing and presentation of antigens [18]. The existence of mTR-Fc molecules might increase uptake of rabbit RK13 cells derived antigens (antigens containing RK/SK cells) into APCs through the FcRs on the surface of APCs, processing, presentation and production of antibodies reacting RK13 cells.

On the other hand, the ligation of Fe portion of IgG and receptor for Fe region of IgG (FcγR) on APCs results in increasing of an anti-inflammatory cytokine, interleukin 10 (IL-10), production by lipopolysaccaride (LPS) activated APCs [1, 4, 14]. To examine whether the mTR-Fc molecule conserves the ability to up-regulate IL-10 production via the ligation with FcγR, mouse splenocytes were co-cultured in the presence of LPS with RK/SK (null) or RK/SK (low) cells and then the production level of IL-10 was measured. The splenocytes harvested from a BALB/c mouse were suspended in RPMI1640 medium containing 10% fetal bovine serum and 5 µg/ml of LPS at the concentration of 10^6 cells/ml. The cell suspension was added into empty wells of 96 wells cell-culture plate or wells of which bottoms confluent mono-layer RK/SK (null) or RK/SK (low) cells were attached to. After 48 hr co-cultivations, IL-10 concentrations in the supernatant were measured by sandwich ELISA system (mouse IL-10 ELISA developing kit, GT &D systems, MS, U.S.A.). It had been confirmed that neither RK/SK (null) nor RK/SK (low) cells produce detectable level of IL-10 without splenocytes (data not shown). As shown in Fig. 3, the supernatant of co-cultivation samples with RK/SK (null) contained IL-10 at the same level as the culture of
The supernatant of co-cultivation sample with RK/SK (high) and parental RK13 cells showed similar result to that of RK/SK (low) and RK/SK (null) cells, respectively (data not shown). Although the expression level of IL-10 was varied among three independent experiments (Fig. 3), when differences of IL-10 production within three independent experiments between samples with RK/SK (null) and RK/SK (low) was tested by paired t-test, there was significant difference (p<0.05). The difference of IL-10 production levels among three mice might be derived from the difference of microbiological environment, because these mice were kept in the conventional area. These results indicate that the mTR-Fc molecule conserves the ability to alter the IL-10 production of splenocytes and/or APC.

IL-10 is known as anti-inflammatory cytokine. It was also suggested that Th-2 biasing immune response are induced via the reciprocal alteration of IL-10 production [1]. However, as shown in Fig. 2B, the immunization with RK/SK (high) cells induced higher level of antibodies. In addition, RK/SK cells immunization up-regulated not only the production of whole IgG but also IgG2a comparing with the immunization with normal RK13 cells, suggesting Th-1 type immune reaction was also up-regulated by the mTR-Fc molecule (data not shown). Although we don’t know the reason of the contradiction, it is also known that the ligation of IgG Fc domain and FcγR increases antigen uptake into APCs, processing, presentation and results in greater level of immune reactions [3, 6, 7, 13, 17, 19]. Among three known Fcγ receptor, FcγRI, FcγRII and FcγRIII, it was reported that FcγRII and FcγRIII contribute to clearance of IgG1-antigen complex [5] and mouse FcgRII and FcγRIII are known as an inhibitory receptor and stimulatory receptor, respectively [2, 5, 15, 20]. In the in vivo experiments, resulting immune reactions might be reflected in the complicated balance of immune inhibitory effect by FcγRII and stimulatory effects FcγRIII.

In this study, it was demonstrated that the artificial chimeric molecule, mTR-Fc, conserves many properties of IgG1 Fc except the ability to activate complement classical pathway. The molecule, mTR-Fc, could be a vaccine adjuvant and a powerful tool to investigate the effect of Fc domain on following immune reactions. Especially, using the mTR-Fc molecules, it is possible to distinguish the direct effects of IgG Fc domain and indirect effects via complement classical pathway on immune reactions.

All animal experiments in this study were carried out under the control of Animal Research Committee in accordance with the Guideline on Animal Experiments at the University of Tokyo and Gifu University.
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