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IL-23 Induces Stronger Sustained CTL and Th1 Immune Responses Than IL-12 in Hepatitis C Virus Envelope Protein 2 DNA Immunization

Sang-Jun Ha,* Doo-Jin Kim,* Kwan-Hyuck Baek,* Yung-Dae Yun,† and Young-Chul Sung2*

IL-23 is a heterodimeric cytokine consisting of p19 and the p40 subunit of IL-12. IL-23 has been shown to possess IL-12-like biological activities, but is different in its capacity to stimulate memory T cells in vitro. In this study, we investigated whether IL-23 could influence envelope protein 2 (E2)-specific cell-mediated immunity induced by immunization of hepatitis C virus E2 DNA. We found that IL-23 induced long-lasting Th1 and CTL immune responses to E2, which are much stronger than IL-12-mediated immune responses. Interestingly, IL-23N220L, an N-glycosylation mutant showing reduced expression of excess p40 without changing the level of IL-23, exhibited a higher ratio of IFN-γ to IL-4-producing CD4+ T cell frequency than did wild-type IL-23, suggesting a negative regulatory effect of p40 on Th1-prone immune response induced by IL-23. These data suggest that IL-23, particularly IL-23N220L, would be an effective adjuvant of DNA vaccine for the induction of durable Ag-specific T cell immunity. The Journal of Immunology, 2004, 172: 525–531.

The hallmark of any successful vaccine is the ability to induce long-term memory. In particular, the memory cell-mediated immune response is a critical determinant for protecting host from chronic infection (1–3). DNA vaccine has been shown to engender relatively long-lived humoral and cellular immune responses in vivo in a variety of animal models compared with other vaccines (1, 4, 5). Recently, it was shown that Ag-specific CD8+ T cells were detectable up to 40 wk after DNA vaccination, although there were no sources of Ag present in the spleen or lymph nodes at 20 days postvaccination (6). However, the level of long-lasting cellular immunity induced by DNA vaccination was often found to be insufficient to protect host from the infection of intracellular pathogens (7–9). Although the generation of memory T cells is the ultimate goal of all types of vaccines including DNA vaccine, how memory T cells are produced after an initial encounter with Ag has been the subject of intense study and debate. Recently, it has been demonstrated that CD8+ T cells can proliferate and differentiate into memory cells in a programmed manner after a single brief encounter with Ag (2, 10, 11), suggesting the existence of a potential key regulatory factor during initial priming event in driving the process for memory CD8+ T cell formation. In addition, recently several reports showed that cytokine, such as IL-15 and IL-7, might be one of the crucial factors for the generation and maintenance of memory CD8+ T cells (11, 12).

IL-12, a heterodimeric cytokine composed of p35 and p40, plays a key role in cell-mediated immune responses, including proliferation of activated T and NK cells, differentiation of CD8+ T cells, and stimulation of hematopoietic stem cells (13). Recently, the novel protein, p19, was identified to bind with p40, generating a cytokine IL-23 that was secreted from activated dendritic cells (DCs) (14). IL-12 and IL-23 share IL-12Rβ1 for binding of p40 common subunit, but require IL-12Rβ2 and IL-23R to trigger signal transduction, respectively (15). Similar to IL-12, IL-23 induces IFN-γ production from activated CD4+ T cells but preferentially stimulates mouse and human memory CD4+ T cells in vitro, which is distinguished from IL-12 (14, 16). In addition to its direct actions on T cells, IL-23 can also act on DCs directly to promote the production of IL-12 and IFN-γ in vitro (17), suggesting the function of IL-23 as an initiator and regulator of T cell-dependent immunity through the modulation of DCs. However, the in vivo effect of IL-23 on the modulation of immune response to foreign Ag remains to be determined.

Free p40 blocks the binding of p70 to its receptor and inhibits IL-12-mediated biological activity (18, 19), suggesting that in vivo production of p40 may be a novel self-regulatory process, which antagonizes the activity of p70. To minimize the in vivo production of p40, there have been a number of studies focusing on the generation of a genetically engineered single chain fusion (20–23). Recently, we also found that the mutation of N-glycosylation site at Asn220 of mouse p40 subunits reduced the secretion of p40 subunit as a natural antagonist of IL-12, and the co-delivery of the mutant IL-12 (IL-12N220L) with plasmid expressing hepatitis C virus (HCV) envelope protein 2 (E2) significantly enhanced long-lasting E2-specific CTL response (24). Because the p40 subunit is also a component of IL-23, it is possible that free p40 blocks the binding of IL-23 to its receptor. However, the possibility that p40

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3 Abbreviations used in this paper: DC, dendritic cell; HCV, hepatitis C virus; scIL, single chain form of IL; E2, envelope protein 2; E2i, E2 truncated; LDA, limiting dilution assay.
subunit may inhibit IL-23-mediated biological activity in vivo was not yet investigated.

In this study, we examined whether coimmunization of plasmid encoding IL-23 with HCV E2 could enhance and maintain E2-specific Th1 and CTL immune responses and compared it with that of plasmid encoding IL-12. We found that IL-23 induced efficiently long-lasting E2-specific CD4+ and CD8+ T cell immunity, which was much stronger than that induced by IL-12, suggesting an in vivo role of exogenous IL-23 in the generation and/or maintenance of long-lasting T cell immunity to foreign Ag. Furthermore, we also found that IL-23N220L, an N-glycosylation mutant reducing the secretion of excess p40 subunit without changing the level of IL-23, exhibited stronger Th1-prone immune response than did wild-type IL-23, suggesting an in vivo regulatory effect of p40 subunit on IL-12-mediated immune response. Therefore, our data suggest that IL-23 or IL-23N220L could be used as an efficient genetic adjuvant in DNA vaccine and gene therapy requiring durable memory T cell immunity.

Materials and Methods

Plasmids

pACP30 vector is composed of CMV promoter, tripartite leader sequence, bovine growth hormone poly(A) tail, and inverted terminal repeat pairs of adenov-associated virus. pACP30-IL-12 construct was built by inserting IL-12 cDNA in pTV2-IL-12 (24) into pACP30 vector. A mouse cDNA encoding IL-23 p19 subunit (14) was isolated from J774 murine macrophages with RT-PCR using two oligonucleotide primers: MSS, CTG AGT CGA CGG GAA CAA GAT GCT GGA TTT CAC AGC and M3E, GAT AGA TAT CTC TTA TCA TGG TAG CCA TGG GAA CC and then inserted into pGEM-T easy vector (Promega, Madison, WI). Thereafter, pACP30-IL-23 was constructed by replacing p35 gene in pACP30-IL-12, with p19 gene. pACP30-IL-23-N220L construct was built by replacing of p40 cDNA in pACP30-IL-12 construct with p40-N220L gene (24). A plasmid encoding single chain form of IL-23, pACP30-scIL-23, was built by linking p19 gene to p40 gene, which were linked with a 45-bp linker encoding the 15-aa (Gly4Ser3) linker, as previously described (20).

Transfection, ELISA, and immunoprecipitation

Transfection of cytokine-expressing plasmids into COS-7 cells was conducted by electroporation as previously described (24). The levels of p70 and p40 were measured by ELISA (R&D Systems, Minneapolis, MN). For immunoprecipitation, transiently transfected cells were labeled for 2 h with [35S]methionine and immunoprecipitated with anti-IL-12p40 Ab (R&D Systems) from cell lysates and supernatants and resolved in nonreducing or reducing the secretion of excess p40 subunit without changing the level of IL-23, exhibited stronger Th1-prone immune response than did wild-type IL-23, suggesting an in vivo regulatory effect of p40 subunit on IL-12-mediated immune response. Therefore, our data suggest that IL-23 or IL-23N220L could be used as an efficient genetic adjuvant in DNA vaccine and gene therapy requiring durable memory T cell immunity.

Table 1. Sequences of E2 peptides

<table>
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<th>Peptides</th>
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<tr>
<td>E2 384-403</td>
<td>STRTV GGTGEG RTTRN FVSSTP</td>
</tr>
<tr>
<td>E2 404-423</td>
<td>ASGPS QKIQL VNNNG SWHIN</td>
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<tr>
<td>E2 414-433</td>
<td>VNNNG SWHIN RTLW CNDSL</td>
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<tr>
<td>E2 424-443</td>
<td>RTLW CNDSL SSGF1 AALFY</td>
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<tr>
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<td>ERMAS CRFDP KFAGQ WGSIT</td>
</tr>
<tr>
<td>E2 464-483</td>
<td>KFAGQ WGSIT YAESS GSDDR</td>
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<td>CGIVP ASQVC GYPCY TFPSP</td>
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<td>GPYCY FTPSP VVVTG TDRSG</td>
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<tr>
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<td>TDVL1 LNNTR PPOQN WFGCT</td>
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</tr>
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Limiting dilution assay (LDA)

LDA for cytotoxicity was performed essentially as previously reported (24) with the following modifications. Splenocytes isolated from mice at the various weeks after single immunization were diluted in 96-well U-bottom plates (2 replicates/dilution), and mitomycin C-treated (25°C) or IL-4 Ab (BD Pharmingen, San Diego, CA). After overnight incubation at room temperature, the plates were blocked with DMEM containing 10% FBS for 1 h. For the assay, the plates were incubated with the culture medium alone. Maximum lysis was obtained by exposing the target cells to 1% Nonidet-P40.

ELISPOT assay

The 96-well filtration plates (MAIPN4550; Millipore, Bedford, MA) were coated with 50 μl of 3 μg/ml rat anti-mouse IFN-γ or IL-4 Ab (BD Pharmingen, San Diego, CA). After overnight incubation at room temperature, the plates were blocked with DMEM containing 10% FBS for 1 h. For the dilution of CD8+ T cells or CD4+ T cells, 102 of splenocytes were incubated with 10 μg of anti-CD8a (clone 53-6.7) or anti-CD4 (clone GK1.5) Ab (BD Pharmingen) in 1 ml of complete RPMI 1640 medium, respectively, for 30 min on ice and washed with complete RPMI 1640 medium. The cells were incubated in 1 ml of rabbit serum complement (Cedarlane Laboratories, Hornby, Ontario, Canada) diluted with complete RPMI 1640
medium (1:15) for 60 min at 37°C. After washing two times with complete RPMI 1640 medium, the cells were applied into plates with E2 peptide pool (1 mg/ml for each peptide) or CT26-high EC2 cells (2 × 10^5 cells/well). After 24 h, the plates were washed and 50 μl of 2 μg/ml biotinylated rat anti-mouse IFN-γ or IL-4 Ab (BD PharMingen) was added and incubated for 3 h at room temperature. After washing the plates, 50 μl of 1:2000 diluted streptavidin-alkaline phosphatase (BD PharMingen) was added. The plates were washed, and then 50 μl of 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium solution (Promega) was added as substrate. When spot color was sufficiently developed, the reaction was stopped by washing the plates with tap water. The numbers of spots were counted using AID ELISPOT Reader System (Autoimmun Diagnostika, Strassberg, Germany). The number of responsive cells was calculated by subtracting the mean number of spots induced in the absence of E2 peptide pool from that in the presence of E2 peptide pool. IFN-γ or IL-4 ELISPOT responses to medium controls were consistently <10% of the response to peptide stimulation (data not shown).

**Results**

Expression of IL-23, IL-23N220L, and scIL-23

Constructs expressing IL-12, IL-23, and its derivatives including IL-23N220L and single chain form of IL-23 (scIL-23) were built in pACP30 vector to compare expression pattern of their subunits (Fig. 1A). After transfection of each construct, the secretion levels of IL-12p40 monomer/homodimer and IL-12p70 heterodimer were examined by using ELISA (Fig. 1B). IL-23 and IL-12 constructs showed a similar level of p40. Relative p40 secretion levels of IL-23N220L and scIL-23 constructs were 5% and 30%, respectively, compared with that of wild-type IL-23 construct. It is worthwhile to note that p40 secretion level of IL-23N220L construct was similar to that of IL-12N220L construct (24), in which N-glycosylation defect at Asn220 residue of p40 reduced the secretion of excess p40 without changing the level of IL-12 heterodimer. Detection of p40 subunit from scIL-23 fusion construct is presumably due to the excision of linking peptide sequences during posttranslational processing of the fusion protein, because the previous report showed that the p40 subunit is produced from scIL-23 fusion construct (21). Expectedly, IL-12p70 secretion was observed only in IL-12 construct but not in IL-23, IL-23N220L, and scIL-23 constructs. Unfortunately, we could not detect IL-23 heterodimeric form in IL-23, IL-23N220L, and scIL-23 constructs because the ELISA system that detects IL-23 was not commercially available yet.

Next, cell supernatants obtained after transfection with cytokine constructs were subjected to immunoprecipitation analysis using a p40-specific Ab to detect a heterodimeric form of IL-23 or IL-23N220L (Fig. 1C). Two or three bands around 70–80 kDa were detected in IL-12, IL-23, and IL-23N220L under nonreducing condition. As expected, the bands of IL-23 (p40 plus p19) were shown to be smaller than those of IL-12 (p40 plus p35) (Fig. 1C, lanes 2 and 3). The bands of IL-23N220L (Fig. 1C, lane 4) were shifted down compared with those of IL-23 due to deglycosylation at Asn220 residue of p40 subunit. It is likely that the expression levels were not significantly different between IL-23 and scIL-23 constructs, based on immunoprecipitation analysis (data not shown). The expression of p19 and p40 subunits from cell lysates was observed under reducing condition (Fig. 1D), indicating that two subunits of IL-23 or IL-23N220L were linked through interdisulfide chain (Fig. 1D, lanes 3 and 4), as observed in the previous report (14).

Enhancement of long-lasting CD8+ T cell immunity by IL-23

To elucidate the in vivo role of exogenous IL-23 in the modulation of Ag-specific T cell immune responses, the effect of IL-23 DNA on E2-specific CTL immune response induced by E2 DNA immunization was examined at various time points (2, 6, 12, 21, 27, and 36 wk postimmunization) and also compared with that of IL-12 DNA (Fig. 2A). At 2 wk, there was no detectable level of CTL activity in all groups, which indicates that DNA vaccine induces a relatively slow CTL response to specific Ag compared with live viral or bacterial vaccines as previously reported (26). At 6 wk, IL-12- or IL-23-coimmunized mice (Gr3 or Gr4) showed slightly higher CTL activity compared with the mice immunized with E2 DNA alone (Gr2), although the difference was not statistically significant. A significant difference of CTL activity between Gr2 mice and cytokine-coimmunized mice (Gr3 and Gr4) was observed after 12 wk postimmunization. Gr2 mice exhibited a remarkably decreased CTL activity during 12–36 wk. Mice coinjected with IL-12 DNA (Gr3) maintained an intermediate level of CTL activity up to 21 wk, and then showed significantly decreased CTL activity after 27 wk. Of interest, IL-23 DNA-coimmunized mice (Gr4) have induced the highest CTL activity with a little decrease up to 36 wk. This result indicates that in vivo coexpression of exogenous IL-23 enhances a long-lasting, sustained E2-specific CTL activity in E2 DNA immunization.

To further analyze the effect of IL-23, the frequency and kinetics of E2-specific CD8+ T cells were determined by LDA (Fig. 2B)
Thus, we performed both LDA and IFN-cytokines (27 cells as well as CD8/H11001 showed an elevated frequency of E2-specific CTL at 6 wk follow-

FIGURE 2. Effect of IL-23 on E2-specific CD8+ T cell response. A, Effect of coimmunization with plasmid expressing IL-12 or IL-23 on E2-specific CTL response. CTL activity was determined by measuring specific lysis of CT26-hghE2t with splenocytes obtained from three mice at the indicated time points. Data are represented as the mean percentage of specific lysis for the indicated E:T ratios in triplicate cultures. This result was reproducible in two independent experiments. B, Frequency and kinetics of E2-specific CTL. Splenocytes from DNA-immunized mice were obtained at the indicated time points. The frequency of precursor CTLs per 10^6 splenocytes was calculated by regression analysis of the number of negative wells at each dilution of responder cells. Data represent the average value of two independent experiments. C, Frequency of IFN-γ-producing CD8+ T cells. Splenocytes from three mice were used in ELISPOT assay to determine the number of cells secreting IFN-γ in response to CT26-hghE2t after depletion of CD4+ T cells. The IFN-γ-producing cell number per 10^6 splenocytes was represented as the average spot forming units (SFU) ± SD in triplicate wells. Statistical significance was determined using the Student t test.

and ELISPOT assay (Fig. 2C). It has been well known that Ag-specific cytolytic activity is a more specific characteristic of CD8+ T cell response, rather than IFN-γ induction activity, because IFN-γ production could be induced from Ag-specific CD4+ T cells as well as CD8+ T cells. However, CD8+ T cells may vary in their effector functions, such as cytotoxicity or production of cytokines (27–30). Thus, we performed both LDA and IFN-γ ELISPOT assay at various time points. Gr2, Gr3, and Gr4 mice showed an elevated frequency of E2-specific CTL at 6 wk follow-

Next, to analyze the effect of in vivo IL-23 expression on Ag-specific Th1 immune response, we performed IFN-γ ELISPOT assay for E2-specific CD4+ T cells at 6, 21, 27, and 36 wk following DNA immunization (Fig. 3A). At 6 wk, the frequency of CD4+ T cells producing IFN-γ was higher in Gr3 and Gr4 than in Gr2, albeit it was statistically insignificant. At this time point, we observed ~50 IFN-γ-positive spots per 10^6 splenocytes of Gr2 mice. Regarding low spot numbers, DNA vector-based immunization has been known to induce a weak Ag-specific immune re-

FIGURE 3. Effect of IL-23 on E2-specific Th immune response. A, Frequency of IFN-γ-producing CD4+ T cells from DNA-immunized mice. Splenocytes from three mice were used in ELISPOT assay to determine the number of cells secreting IFN-γ in response to E2 peptide pool after depletion of CD8+ T cells. The IFN-γ-producing cell number per 10^6 splenocytes was represented as the average spot forming units (SFU) ± SD in triplicate wells. Statistical significance was determined using the Student t test. B, Ratio of IFN-γ to IL-4-producing CD4+ T cell frequency. Splenocytes from three mice at 36 wk after DNA immunization were used to determine the ratio of cells producing IFN-γ or IL-4 after depletion of CD8+ T cells. The IFN-γ or IL-4-producing cell number per 10^6 splenocytes was represented as the average SFU ± SD in triplicate wells and the values at top of bars represent the ratio of IFN-γ/IL-4 SFU. These results were reproducible in two independent experiments.

result indicates that generation of E2-specific CTL memory in-

creases during 2–6 wk after DNA immunization, followed by con-

traction between 6–21 wk. A similar pattern of precursor CTL frequency kinetics was observed between Gr2 and Gr3, although the CTL frequency of Gr3 was continuously higher than that of Gr2. Coimmunization of IL-23 DNA (Gr4) showed very stable maintenance of memory E2-specific CTL frequency during 12–36 wk, suggesting that coimmunization of IL-23 DNA may induce prolonged, sustained CTL immunity by maintaining memory CTL specific to co-delivered Ag. These patterns were also observed similarly in E2-specific IFN-γ ELISPOT assay for CD8+ T cells at 6, 21, and 27 wk after DNA immunization (Fig. 2C). However, the frequency of IFN-γ-producing CD8+ T cells was approximately five to seven times higher than that of cytotoxic CD8+ T cells obtained from LDA, which is presumably due to a relatively insensi-

itive LDA with a cytotoxicity readout.

Enhancement of long-lasting Th1 immune response by IL-23

Maintenance of long-term E2-specific immunity was determined by measuring IFN-γ by ELISPOT assay for CD8+/H11001 cells from three mice at 36 wk after DNA immunization were used to determine the number of cells secreting IFN-γ in response to E2 peptide pool after depletion of CD8+ T cells. The IFN-γ-producing cell number per 10^6 splenocytes was represented as the average SFU ± SD in triplicate wells. Statistical significance was determined using the Student t test.

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than E2 DNA-immunized mice (Gr2), indicating that IL-12 induced a long-lasting Th1 immune response. Interestingly, IL-23 DNA-coimmunized mice (Gr4) showed even higher E2-specific IFN-γ frequency, than Gr2 and Gr3 mice did. This observation demonstrates that in vivo expression of IL-23 affects the generation of long-lasting Th1 immune response. In addition, it seems that IL-23 may have a stronger Th1-inducing activity than IL-12 in DNA vaccination.

To investigate the type of Th immune response, ELISPOT assay for IL-4 secreted from CD4⁺ T cells was also performed at 36 wk and the ratio of IFN-γ- to IL-4-producing CD4⁺ T cell frequency was examined (Fig. 3B). Mice coimmunized with IL-23 DNA (Gr4) showed higher IFN-γ- and IL-4 frequencies than Gr2 and Gr3 mice did. Thus, the ratio of IFN-γ to IL-4 was similar between Gr3 and Gr4. These results indicate that IL-23 induces stronger Th immune responses than IL-12 does, but similar Th1-prone immune responses compared with IL-12.

Effect of p40 on cell-mediated immunity enhanced by IL-23

Our results demonstrated that IL-23 could efficiently enhance long-lasting Ag-specific T cell-mediated immune response. Because excess p40 can bind to IL-12Rβ1, which is a common receptor of IL-12 and IL-23, it is possible that it blocks the binding of IL-23 to its receptor and inhibits IL-23-mediated biological activity. In the case of IL-12, we previously demonstrated that excess p40 inhibits IL-12-mediated long-lasting Th1 and CTL immunity through comparing T cell immunity observed after coimmunization of IL-12 DNA or IL-12N220L DNA with E2 DNA. These two constructs are different only in the secretion level of p40 subunit, but similar in receptor binding affinity and in vivo stability. In this regard, we became interested in the possibility that excess p40 could also inhibit the induction of long-lasting T cell immunity mediated by IL-23. At 27 wk after DNA immunization, we compared the effect of IL-23 with that of IL-23N220L or scIL-23 in enhancing long-lasting T cell immune responses (Fig. 4). It is worth noting that they are similar in the expression level of heterodimeric IL-23, but significantly different in the secretion level of p40 (100%, 5%, and 30%, respectively) as shown in Fig. 1. Interestingly, IL-23 and IL-23N220L constructs exhibited similar CTL activity (Fig. 4A) and IFN-γ-producing CD4⁺ T cell frequency (Fig. 4B), indicating that excess p40 does not interfere with long-lasting CTL and Th1 immune responses enhanced by coinjection of IL-23. As expected, CTL and Th1 immune responses induced by scIL-23 were significantly lower than those induced by IL-23 (Fig. 4, A and B), presumably due to a reduced specific activity of single chain form caused by an altered structural change. This result may be explained by the recent report that the in vitro bioactivity of scIL-12 protein is lower than that of native heterodimeric IL-12 protein with regard to IFN-γ production (22, 23). However, IL-23N220L and scIL-23 showed higher a ratio of IFN-γ to IL-4 than IL-23. These results suggest that the presence of excess p40 subunit may increase IL-4-producing CD4⁺ T cell frequency, leading to a negative impact on IL-23-mediated Th1-prone immune response. This possibility is further supported by our unpublished result that coimmunization of p40-expressing plasmid with E2 DNA showed higher frequency of IL-4-producing CD4⁺ T cells than did the immunization of E2 DNA alone (data not shown).

Discussion

In this study, we addressed the effect of IL-23 on long-lasting CD8⁺ and CD4⁺ T cell responses specific to coadministered E2 Ag. In primary response to Ag, T cells undergo a massive burst of expansion followed by a contraction in which the majority of Ag-specific T cells undergo apoptosis, leaving behind a stable memory population. DNA vaccination as well as natural infections caused by viruses and bacteria also displays the Ag-specific T cell kinetics such as expansion, contraction, and memory phase (5, 26, 33). Akbari et al. (6) reported that vaccination of DNA construct encoding nonsecreted fifth component of complement (C5) protein induced the peak response of C5-specific memory CD4⁺ T cells at 4–6 wk after DNA immunization, and allowed these CD4⁺ T cell responses to persist for longer than 40 wk despite the absence of a source of persistent Ag. Our results showing that kinetics of E2-specific CD8⁺ (Fig. 2) and CD4⁺ T cells (Fig. 3) after immunization of E2 DNA (Gr2) appeared to agree well with the previous report (6). Thus, our E2 DNA immunization model is an appropriate system to investigate whether coimmunized cytokine genes can affect the generation of long-lasting Ag-specific T cells induced by Ag produced by plasmid DNA.

It was reported that IL-12 appeared to be effective for stimulating clonal expansion and establishment of a long-lived memory population of CD8⁺ T cells in the presence of TCR interaction with peptide/MHC (34, 35) and coadministration of IL-12 DNA or protein with Ag increased long-lived memory Th1 (36, 37) and CTL responses (24, 38). Regarding IL-23, it was shown that in vitro treatment of IL-23 could induce strong proliferation of memory T cells (14) and activate memory T cells for production of the proinflammatory cytokine IL-17 (16). However, there were no reports about its ability to enhance in vivo T cell immunity to foreign Ag, although Cua et al. (39) recently demonstrated using IL-23 p19 KO mice the function of endogenous IL-23 in causing autoimmune inflammation of the brain through direct actions on macrophages in vivo. Thus, our present data are important in the point that in vivo expression of exogenous IL-23 DNA could significantly enhance long-lasting memory T cell immunity such as E2-specific CTL (Fig. 2) and IFN-γ-producing Th1 immune responses (Fig. 3).

In an E2-specific T cell differentiation process induced by E2 DNA immunization, co-delivery of IL-23 DNA appears to affect late phase of CD8⁺ T cell immune response rather than early phase, because the frequency of E2-specific CTL was sustained...
until at least 36 wk by co-delivery of IL-23 DNA. It is worth noting that the quantity of E2-specific CTL at 6 wk was less dependent on the presence of IL-23 (Fig. 2B). This result suggests that IL-23 expressed during immune-induction phase may alter the quality rather than the quantity of CD8+ T cells, thereby eventually contributing to the long-term establishment of Ag-specific memory T cells. Consistent with our data, several recent reports indicated that the number of long-lived memory CD8+ T cells depends more on the quality of CD8+ T cells rather than the burst size of effector cells (40, 41). In addition, the cytokine milieu during the priming may be one of the major factors that determine the life and quality of memory CD8+ T cells. Also, with regard to memory CD4+ T cell response, the effect of co-delivered IL-23 was clearly observed in a late phase rather than an early phase of immune response induction (Fig. 3A), suggesting that IL-23 may play a role in the long-term establishment of memory CD4+ T cells in early phase of immune response.

Mouse memory CD4+ CD45RBlow cells respond to IL-23 but poorly to IL-12, whereas naive CD4+ CD45RBhigh cells respond to these cytokines in reverse manner (14). It is also correlated with the reports that IL-23R is expressed by CD4+ CD45RBlow cells but less by CD4+ CD45RBhigh cells and IL-12Rβ2 shows the reciprocal expression pattern (15). Thus, it is possible to speculate that IL-23 and IL-12 may act on a different phenotype of T cells during immune-induction phase. IL-12 may act on activated T cells during expansion of Ag-specific T cells, and contribute to reprogramming these T cells into memory cells by increasing the rate of survival or proliferation (42–45), which might eventually increase Ag-specific T cell numbers. In contrast, IL-23 might act on Ag-specific memory T cells generated after priming process. However, it is unclear whether IL-23 can act only on memory phenotype of T cells, because IL-23 was also reported to stimulate proliferation of blast T cells obtained after prolonged stimulation (14, 17). Collectively, these observations can lead to a hypothesis that IL-23, like IL-12, can act on T cells activated during the initial exposure of Ag, thereby resulting in the significant increase of Ag-specific memory T cells through increasing survival of these cells, and furthermore act on memory T cells contributing to homeostatic expansion of memory T cells during memory phase like IL-15 or IL-7. However, more detailed experimentation will be needed to clarify the mechanism by which IL-23 can contribute to the generation of Ag-specific memory T cells.

Tough et al. (46) recently reported that IL-12 injection could indirectly stimulate memory phenotype CD8+ T cell turnover in vivo through an IFN-γ-dependent pathway. Because IL-12 was unable to induce proliferation of purified T cells in vitro despite its strong stimulatory effects in vivo (46), it is likely that the in vivo function of IL-12 may be caused by IL-12-mediated IFN-γ production from DCs, which constitutively express IL-12Rβ1 and β2 (47, 48), followed by IFN-γ-induced IL-15 production (49). Similarly, IL-23 may induce homeostatic expansion of memory T cells in IFN-γ-dependent manner produced from macrophages and DCs, because IL-23R is also constitutively expressed on mouse macrophages and DCs (15, 17, 39) and IL-23 can induce IFN-γ and IL-12 production from DCs (17).

In this study, we demonstrated that coinoculation of IL-23 increased the frequency of both IFN-γ- or IL-4-producing CD4+ T cells, suggesting that IL-23 is involved in the generation of Th2 cells as well as Th1 cells. Consistent with our data, Cua et al. (39) observed that there was little or no IL-4 production after in vitro stimulation of lymph node cells from p19 knockout mice, which demonstrates that IL-23 is involved in the generation of Th2 immune response. However, we found that coinoculation of IL-23N220L DNA induced a higher IFN-γ- or IL-4 CD4+ T cell frequency ratio than did that of IL-23 DNA, suggesting that the role of intact IL-23 in the absence of excess p40 might be the induction of Th1-prone immunity rather than Th2-prone immunity. In addition, it is likely that IL-23 is able to enhance both Th1 and Th2 immune response and that excess p40 confer a negative regulatory effect on Th1-prone immune response induced by IL-23. Interestingly, CTL activity and frequency of IFN-γ-producing CD4+ T cells were similar between IL-23- and IL-23N220L-immunized mice (Fig. 4), indicating that excess p40 has no effect on the induction of CTL activity and IFN-γ production, but enhances IL-4 production, thereby leading to inhibit the establishment of IL-23-mediated Th1-prone immunity. To verify this, it will be required to closely investigate the effect of p40 on IL-23 signaling pathway.

In this study, we demonstrated that coinoculation of IL-23 DNA enhanced long-lasting cell-mediated immunity including CTL and Th1, suggesting that IL-23 might be involved in the induction of sustained memory CD4+ T cell immune response in vivo. In addition, the CTL immune response induced by IL-23 was much more durable than that by IL-12. The expression of excess p40 appeared to confer a negative effect on IL-23-mediated Th1-prone immunity generation. These data suggest that N-glycosylation mutant of IL-23, IL-23N220L, could be used as an effective genetic adjuvant in maintaining long-term memory CD4+ and CD8+ T cell responses specific to co-delivered Ag, in particular Th1-prone immunity in DNA vaccination.

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References


