The Actions of Polynucleotides on Effector Stage Cloned Murine T-Helper Cells Differ in Each Subset and Depend on Antigen Concentration1,2

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ABSTRACT. Polynucleotides enhance T-helper (Th) cell–mediated humoral immune responses in naive resting Th cells, B cells, and antigen-presenting cells (APC) from unprimed mouse spleen. If polynucleotides augment Th cell functions independent of the activation stage of Th cells, then polynucleotides may cause hyperimmune responses. In this study we examined the effects of polynucleotides on effector-stage murine Th cell clones in vitro. The A.E7 clone (primed with pigeon cytochrome C, origin: B10.A mice) and CDC35 clone (primed with rabbit γ-globulin, origin: DBA/2 mice) were used as representative type 1 (Th1) and type 2 (Th2) Th cells, respectively. Th clones were stimulated with antigen (Ag) in polynucleotide-supplemented or control cultures in the presence of syngeneic spleen cells (either CD4+ or irradiated). The number of antibody (Ab)-secreting cells was counted to measure T-dependent Ab production. Production of interferon-γ (IFN-γ) for the Th1 clone and interleukin-5 (IL-5) for the Th2 clone were measured. Without Ag stimulation, cytokine production and the number of Ab-secreting cells formed were very low and not altered by polynucleotides. With suboptimal Ag challenges provided by Ag-primed spleen cells, polynucleotides enhanced IFN-γ production by the Th1 clone, while they suppressed Th1 clone–mediated Ab production and IL-5 production by the Th2 clone. Polynucleotides did not alter Th2 clone–mediated Ab production. These actions of polynucleotides appeared to be dose-dependent. With optimal Ag challenges, polynucleotides did not affect our measures of Th cell activation. Polynucleotide action in vitro on effector-stage Th cell clones differed in each Th cell subset and depended on Ag concentration. J. Nutr. 127: 411–417, 1997.

KEY WORDS: • polynucleotides • T-helper cells • antibody-secreting cells • cytokines • immune response • mice

Prolonged deprivation of dietary nucleotides appears to suppress the immune response. Mice fed a nucleotide-free diet for more than two weeks demonstrate decreased cellular and humoral immunity (Jyonouchi 1994, Rudolph et al. 1990, Van Buren et al. 1994). These mice easily succumb to death caused by bacterial and fungal pathogens (Adjei et al. 1992, Fanslow et al. 1990, Kulkarni et al. 1986). Nevertheless, the mechanisms of nucleotide action on the immune system are not well understood, which makes it difficult to understand the role of dietary nucleotides.

The immune system protects an individual by developing antigen (Ag)-specific immune responses. Previously we analyzed nucleotide action on Ag-mediated immune responses. We have shown in vivo and in vitro that nucleotides augment antibody (Ab) production against T-dependent Ag in mice (Jyonouchi et al. 1992, 1993a, 1994). This enhancement has been demonstrated in several normal strains of mice, and the degree of enhancement was independent of major histocompatibility complex (MHC) restriction (Jyonouchi et al. 1993b) and was largely attributed to polynucleotides (Jyonouchi et al. 1994, Jyonouchi et al. 1995a, 1995b). Our results indicate that polynucleotides augment T-dependent Ab production by modulating processes of Ag-mediated T-helper (Th) cell activation (Jyonouchi, et al. 1993a, 1995b). The actions of polynucleotides likely occur at the local site of inflammation when injured cells release polynucleotides. Polynucleotides may augment activation of naive Th cells and amplify local immune responses.

When Ag are presented by Ag-presenting cells (APC), naive Th cells differentiate into at least three types of effector-stage Th cells: type 0, type 1 and type 2 Th cells (Th0, Th1 and Th2, respectively), differentiated on the basis of cytokine production pattern (Romagnani 1994, Seder and Paul 1994, Swain et al. 1991). The effector-stage Th cells are more easily activated than resting Th cells (Croft 1994) and are much more active in cytokine production and other Th cell activities. If polynucleotides enhance effector-stage Th cells nonspecifically, this could lead to potentially hazardous, hyperactive immune responses. However, we found that nucleotides do not augment polyclonal B cell activation or T cell responses potentiated by polyclonal stimulators (Jyonouchi et al. 1992, 1993b, 1994a). We thus hypothesize that polynucleotide ef-
ffects differ depending on the stage of Th cell activation. In this study we examined polynucleotide action on effector-stage Th1 and Th2 cell clones.

The use of cloned Th cell lines enables us to examine the effects of polynucleotides in each Th cell subset in a quantitative manner. Fewer than 0.3% of Th cells isolated from regional draining lymph node cells following in vivo Ag challenge are Ag-specific, and those consist of mixed populations of Th0, Th1, and Th2 cells (Miller 1991, Seder and Paul 1994, Swain 1991). In contrast, the two cloned Th cell lines used in this study were each developed from a single primed Th cell, are Ag-specific, and each produces a large amount of cytokines characteristic of a Th subset (Paul and Seder 1994 and Swain et al. 1991). The use of cloned Th1 and Th2 cells permits us to greatly increase the proportion of Ag-specific Th cells in the assay as compared with primary cultures of Ag-primed cells. This system could be used a model for elucidating polynucleotide action at the molecular level.

MATERIALS AND METHODS

Mice. Female mice of the strains B10.A and DBA/2 were 5–6 wk of age when received (Jackson Laboratories, Bar Harbor, ME) and were maintained in the animal facility at the University of Minnesota, Minneapolis, MN. Mice were housed in groups of five per cage and fed a nonpurified diet* (Purina Lab Chow #5008, Purina Mills, Richmond, IN). Mice were killed in a CO2 chamber as approved by the Laboratory Animal Medicine Ethics Committee of the University of Minnesota. Mice were 2- to 4-mo-old when used in the experiments.

Cell suspensions. Spleen cell suspensions were prepared by crushing the spleen between two sterile glass slides and suspending cells in RPMI 1640 medium supplemented with 50 mL heat-inactivated fetal calf serum (HI-FCS). The spleen cell suspensions were depleted of CD4+ Th cells by treatment with monoclonal rat anti-mouse L3T4 Ab (specific for CD4, Dikins et al. 1982) and guinea pig complement (Pel-Freeze, Rogers, AR). More than 98% of CD4+ cells were removed by this treatment as determined by flow cytometry (FACS Scan, Beckton Dickinson, San Jose, CA). Monoclonal anti-L3T4 Ab was obtained from hybridoma cell line GK1.5 (American Type Culture Collection, Rockville, MD). culture supernatant purified in our laboratory.

Polynucleotides. FDA-certified yeast RNA preparations were a gift from Sandoz Nutrition Corp. (Minneapolis, MN). The components of the RNA preparations were: RNA, 910 g/L; hydrolysate of RNA, 50 g/L; cold acid–soluble fraction including sodium salts of mononucleotides, 10 g/L; sugars, 5 g/L; and NaCl, 25 g/L. The RNA preparations were shown to be endotoxin-free and consistently effective in augmenting in vitro Ab production (Jyonouchi et al. 1992, 1994, 1995a). The RNA preparations were already degraded to small polynucleotides, evenly distributed between 300 and 1000 nucleotides on gel electrophoresis. The enhancing actions of polynucleotides were not lost when the RNA preparations were dialyzed (molecular weight cut off 1000 or 10000) (Jyonouchi et al. 1992 and unpublished observations). Treatment with polymyxin B (endotoxin inhibitor), DNase, trypsin, and phenol extraction for protein did not alter the effects of the RNA preparations but modulation of RNA greatly diminished their actions (Jyonouchi et al. 1992 and 1995a). The RNA preparations were kept at −20°C in lyophilized form until the day of the experiment when they were dissolved in double distilled, autoclaved water.

Maintenance of Th cell clones. Cell line A.E7, a type Th1 clone developed from a B10.A mouse (MHC type I-E5) immunized with pigeon cytochrome C peptide (94–103), was kindly provided by Marc K. Jenkins, Department of Microbiology, University of Minnesota (Jenkins et al. 1987, Weaver et al. 1988). The A.E7 cells were maintained in our laboratory in Dulbecco’s Modified Eagle Medium supplemented per liter with 100 mL HI-FCS (HyClone), 107 u penicillin, 100 mg streptomycin and 10−5 mol 2-mercaptoethanol. Cultures were stimulated every 2–3 wk with Ag (pigeon cytochrome C; 3 µmol/L, Sigma, St. Louis, MO) and irradiated (3000 rad) B10.A spleen cells. Cell line CDC35, a Th2 clone developed from a DBA/2 mouse (MHC type I-A²) immunized with rabbit γ-globulin, was kindly provided by David Parker, Department of Molecular Genetics and Microbiology, University of Massachusetts, Worcester, MA (Boom et al. 1988, Tony et al. 1985). The CDC35 cells were maintained in our laboratory in the same medium used for A.E7 cells and were stimulated every 2–3 wk with Ag (rabbit γ-globulin; 25–50 mg/L, Sigma) and irradiated (3000 rad) DBA/2 spleen cells.

In vivo Ag-priming. Mice were twice injected subcutaneously at 2–3 wk intervals with pigeon cytochrome C (25 µmol/L) for B10.A mice and rabbit γ-globulin (50 µg/L) for DBA/2 mice. Each injection was a 0.5-mL dose of specific Ag suspended in a mixture of 1 part PBS and 1 part Freund’s complete adjuvant. A 0.25-mL booster of the same Ag concentration suspended in PBS was given intraperitoneally 5–7 d prior to the experiment (Bradley et al. 1993). Spleen cells from in vivo immunized B10.A or DBA/2 mice were used as the source of primed B cells and APC.

ELISPOT assay for IgM and IgG antibody-secreting cells. Numbers of Ag-secreting cells were measured by enzyme-linked immunosorbent ELISPOT (ELISPOT) assay (Sedgwick et al. 1985). Cloned Th cells were stimulated with Ag-coated splenic cells; Ag-coated well (2 × 107 cells/L) with CD4+ B10.A spleen cells (1.25 × 107 cells/L), or CDC35 cells (2.5 × 107 cells/L) with CD4+ DBA/2 spleen cells (2 × 107 cells/L). This ratio of APC to Th clones produced the optimal results in this assay. Cells were incubated for 5 d in the same medium used for maintaining Th clones in a 24-well tissue culture plate (Costar, Cambridge, MA). The wells were harvested, counted, and resuspended in Iscove’s Modified Dulbecco’s Medium supplemented per liter with 50 mL HI-FCS, 107 U penicillin, 100 mg streptomycin and 2 mmol glutamine. The harvested cell mixture (100 µL/well) was incubated overnight at 37°C in a 5% CO2 incubator in a 96-well microriter plate (Costar) coated with Ag (pigeon cytochrome C; 5 µmol/L) and preincubated with a blocking buffer (PBS, pH 7.4 with 10 g bovine serum albumin/L) for 30 min at 37°C. Cells were removed the next day by vigorous washing, and goat anti-mouse IgG or IgM alkaline phosphatase–alkaline phosphatase conjugate (1:1000 in dilution buffer used for ELISA; 100 µL/well, Sigma) was added to each well. The plate was incubated overnight at 4°C and washed again. Abs secreted by cells were detected by adding gel substrate solution [5-bromo-4-chloro-3-indolyl phosphate (75 mg/L), and nitroblue tetrazolium chloride (150 µg/L) in 50 mL/mL NaHCO3, pH 9.8 with MgCl2 (5 mmol/L) and agarose (5 g/L, agarose Type 1-A; low EEO, Sigma), 100 µL/well] and incubating the plate at 37°C for 4–5 h. Triplicates were tested for each sample, and results are expressed as mean number per 105 viable cells based on the cell count at 5 d culture. This culture period provided the optimal responses and cell viability declined when cells were cultured more than 6 days. When CD4+ spleen cells were not reconstituted by Th clones, no ELISPOT was detected (this served as a negative control). The cell mixture cultured without Ag stimuli also served as control for ELISPOT assay.

ELISA for IFNγ and IL-5. Co-cultures of A.E7 cells and irradiated B10.A spleen cells (3000 rad) (final cell concentration: 1.45 × 107 cells/L), or CDC35 cells and irradiated DBA/2 cells (3000 rad), were incubated in 96-well tissue culture plates with 100 mL PC580. Cytokine levels in the harvested supernatants were measured by enzyme-linked immunosorbent assay (ELISA). These ratios of Th clones and spleen cells produced the optimal results for this assay in preliminary experiments. Three day incubation time was also optimal for IL-5 and IFNγ production by the Th clones. We used unconjugated anti-mouse IFNγ and IL-5 [2–3 µg/mL in the coating buffer (0.1 mol/L NaHCO3, pH 8.3), Pharmingen, San Diego, CA] as the first Ab and biotinylated anti-mouse IFNγ and IL-5 [2–3 µg/mL, Sigma, St. Louis, MO] as the second Ab. ELISA plates (Nunc, Naperville, IL) were coated with the first Ab overnight at 4°C, then samples were
RESULTS

Experiment 1: Polynucleotide action on Th cell clone-mediated Ab production. Numbers of IgM Ab-secreting cells were unaffected by supplemental polynucleotides in both Th1 and Th2 clones (Fig. 2 and data not shown). The numbers of IgG Ab-secreting cells were fewer than 1/105 viable cells in two trials. Supplemental polynucleotides did not alter the number of IgG Ab-secreting cells compared to controls (data not shown). The number of IgM Ab-secreting cells was lower in the polynucleotide-supplemented cultures than controls at cytokine concentrations of 0.25, 0.5 and 1 μmol/L in the Th1 clone (Fig. 3-A). The number of IgG Ab-secreting cells was also lower in cultures with polynucleotide supplementation than in control cultures at cytokine concentrations of 0.25, 0.5 and 1 μmol/L in the Th1 clone (Fig. 3-B). At higher, optimal Ag concentrations, polynucleotides did not significantly alter the number of Ab-secreting cells. This sup-

![Th cells and unprimed spleen cells](image)

FIGURE 2 Numbers of IgM antibody (Ab)-secreting cells in response to various doses of cytochrome C. Type 1 T-helper cells and CD4+ B10. A spleen cells were cultured for 5 d, and numbers of Ab-secreting cells were detected by ELISPOT assay. Spleen cells were obtained from untreated B10.A mice (unprimed spleen cells). The cells were cultured with polynucleotides (0.1 g/L) or without polynucleotides (control). Values are means ± SD, n = 5.
cells, IFN-γ production by A.E7 cells was higher in the polynucleotide-supplemented cultures than in control cultures at a cytochrome C concentration of 1 μmol/L (P < 0.05, data not shown). When in vivo Ag-primed spleen cells were used, polynucleotides significantly augmented IFN-γ production at cytochrome C concentrations of 0.5 and 1 μmol/L (Fig. 5). This enhancing action was observed with 0.1 and 0.01 g/L polynucleotides but not with 0.001 or 0.0001 g/L of polynucleotides (Fig. 6). Enhancement of IFN-γ production by nucleotides was evident when they were present from day 0 of the culture but not when they were added to the culture on day 1 or later (data not shown). Without Ag (cytochrome C), IFN-γ production by A.E7 cells was not altered by polynucleotide supplementation (Fig. 5).

IL-5 production by CDC35 cells was not affected by polynucleotides when unprimed spleen cells were used (data not shown). With the use of in vivo Ag-primed spleen cells, less IL-5 was produced with polynucleotide supplementation than in controls at rabbit γ-globulin concentrations of 2.5 and 5 mg/L (Fig. 7). This suppressive action of polynucleotides appeared to be concentration-dependent at rabbit γ-globulin concentrations of 2.5 mg/L (Fig. 8) and 5 mg/L (data not shown). This action of polynucleotides was observed only

![FIGURE 3](https://example.com/figure3)

**FIGURE 3** Numbers of IgM (panel A) and IgG (panel B) antibody (Ab)-secreting cells in response to pigeon cytochrome C with the use of primed B10.A spleen cells. Type 1 T-helper (Th1) cells and CD4+ B10.A spleen cells were cultured for 5 d and numbers of Ab-secreting cells were detected by ELISPOT assay. Spleen cells were obtained from vivo antigen (cytochrome C)-treated B10.A mice (primed spleen cells). The cells were cultured with polynucleotides (0.1 g/L) or without polynucleotides (control). Values are means ± SD, n = 6. *Significantly different from control values, P < 0.05. **Significantly different from control values, P < 0.01.

pressive action of polynucleotides appeared to be dose-dependent when cells were stimulated with 1 μmol/L of cytochrome C (Fig. 4). Polynucleotides were effective only when they were present from day 0 of the culture, consistent with our previous results in T-dependent Ab production assays (Jyonouchi et al. 1993a). We obtained similar results in two experiments with A.E7 cells stimulated at a cytochrome C concentration of 0.5 μmol/L. In cultures with the Th2 clone, polynucleotide supplementation did not alter the number of Ab-secreting cells (data not shown).

**Experiment 2: Polynucleotide action on IFN-γ and IL-5 production by the cloned Th cells.** With unprimed spleen

![FIGURE 4](https://example.com/figure4)

**FIGURE 4** Numbers of IgM (panel A) and IgG (panel B) antibody (Ab)-secreting cells in response to various doses of polynucleotides at a cytochrome C concentration of 1 μmol/L. Type 1 T-helper (Th1) cells and CD4+ B10.A spleen cells were cultured for 5 d and numbers of Ab-secreting cells were detected by ELISPOT assay. Spleen cells were obtained from in vivo antigen-primed B10.A mice. Values are means ± SD, n = 5. *Significantly different from control values, P < 0.02 by Student's t-test. **Significantly different from controls, P < 0.05.
Th1 cells and primed spleen cells

Th2 cells and primed spleen cells

**FIGURE 5** Interferon-γ (IFNγ) levels in the culture supernatants when A.E7 cells were stimulated by irradiated, in vivo antigen (Ag)-primed B10.A spleen cells and various doses of cytochrome C with supplemental polynucleotides (0.1 g/L) for 3 d. Control cells were cultured without nucleotide supplementation. Values are means ± sd, n = 6. *Significantly different from control values, P < 0.01. **Significantly different from controls, P < 0.05.

when they were present from day 0 of the culture (data not shown).

**DISCUSSION**

Nucleotides exert a variety of immunomodulating actions (Carver et al. 1994, Jyonouchi 1994, Kulkarni et al. 1994, Van Buren et al. 1994). The mechanism of immunomodulation by nucleotides may be attributed in part to their serving as substrate for a salvage pathway of RNA and DNA synthesis (Rudolph et al. 1990). Although <5% of dietary nucleotides are integrated into the tissue nucleotide pool in healthy individuals (Sonoda et al. 1978), exogenous nucleotides may be more actively utilized in the body under certain circumstances; DNA and RNA synthesis through a salvage pathway requires much less energy than does de novo synthesis.

**FIGURE 6** Interferon-γ (IFNγ) production by A.E7 cells with various concentrations of polynucleotides. A.E7 cells were stimulated with irradiated, in vivo Ag-primed B10.A spleen cells and cytochrome C (1 μmol/L) for 3 d and IFNγ levels in the culture supernatants were examined (polynucleotides 0.0001 to 0.1 g/L). Values are means ± sd, n = 5. *Significantly different from control values, P < 0.02 by Student’s t-test. **Significantly different from controls, P < 0.01.

**FIGURE 7** Interleukin-5 (IL-5) levels in the culture supernatants when CDC35 cells were stimulated by irradiated, primed DBA/2 spleen cells and rabbit γ-globulin with supplemental polynucleotides (0.1 g/L) for 3 d. Control cells were cultured without nucleotide supplementation. Values are means ± sd, n = 5. *Significantly different from control values, P < 0.01. **Significantly different from controls, P < 0.05.

**FIGURE 8** Interleukin-5 (IL-5) production by CDC35 cells with various concentrations of polynucleotides. CDC35 cells were stimulated with irradiated, primed DBA/2 spleen cells and rabbit γ-globulin (2.5 mg/L) for 3 d and IL-5 levels in the culture supernatants were examined (polynucleotides 0.0001 to 0.1 g/L). Values are means ± sd, n = 5. *Significantly different from control values, P < 0.05.
Nucleotides also exert more specific actions on the immune system. We have shown that nucleotides preferentially affect T cell-mediated humoral immunity both in vivo and in vitro (Jyonouchi 1994, Jyonouchi et al. 1992, 1993a, 1994). This enhancing action in vitro is attributed to polynucleotides but not to mononucleotides or nucleotide metabolites, while oral supplementation of both polynucleotides and a mononucleotide mixture prevented decline of humoral immunity and Ag-mediated cytokine production in mice fed a nucleotide-free diet (Jyonouchi et al. 1992, 1994, 1995b, 1996). Polynucleotides must be present from day 0 of the culture in order to fully exert their actions (Jyonouchi et al. 1993a). They may modify Ag-mediated T cell activation in the initial stages of Ag presentation. We hypothesized that such polynucleotide action can take place in vivo, most likely at the site of inflammation or tissue injury. That is, the injured tissue could release polynucleotides which may temporarily reach a high local concentration and modulate local immune responses. In mice, deprivation of dietary nucleotides leads to the preservation of DNA at the expense of cellular RNA and proteins, resulting in depletion of the tissue nucleotide pool (Leleiko et al. 1987). Under such conditions, injured tissues release less nucleotides, which may result in attenuated Th cell activation. Dietary nucleotides may be important in maintaining tissue nucleotide pools that may initiate effective local immune responses in certain circumstances.

Naive Th cells generally require more potent signals for activation (Croft 1994), and thus the enhancing action of polynucleotides may promote primary immune responses. Once activated, naive Th cells differentiate into either Th0, Th1, or Th2 effector-stage Th cells (Paul and Seder 1994, Swain et al 1991). Th0 cells produce a variety of cytokines, but Th1 and Th2 cells have a limited pattern of cytokine production (Th1 cells; IL-2 and IFNγ, Th2 cells; IL-4, IL-5, IL-10, and IL-13). Th1 cells are vital in phagocytic cell-mediated, cellular and proinflammatory immune responses, while Th2 cells promote B cell-mediated humoral immune responses and down-regulate inflammatory responses (Garside and Mowat 1995, Paul and Seder 1994). The effector-stage Th1 and Th2 cells produce 100-fold more cytokines than do naive Th cells and are activated more easily and swiftly upon the exposure to the same Ag (recall Ag) than naive Th cells (Croft 1994, Swain et al. 1991). If polynucleotides augment Th cell activation independent of differentiation stages of Th cells, polynucleotides could be hazardous due to induction of hyperactive immune responses. This study was designed to evaluate the actions of polynucleotides on activated, effector stage Th cells using cloned Th cells. The use of cloned Th cells also permitted us to study nucleotide action in Th1 and Th2 cell subsets.

Our results demonstrated that the actions of the polynucleotides differ between effector Th cells and naive Th cells and even in each Th cell subset. We found that polynucleotides suppress Th1 clone-mediated Ab production when Ab production was assessed by the number of Ab-secreting cells. This suppressive action was shown with the use of in vivo Ag-primed spleen cells and appeared to be dose-dependent. Polynucleotides did not augment Th2 clone-mediated Ab production. With suboptimal Ag challenges, polynucleotides augmented IFNγ production by the Th1 clone but suppressed IL-5 production by the Th2 clone in a dose-dependent manner. With optimal Ag challenges, no significant effects of polynucleotides were observed.

Activation of Th cells generally requires at least two signals, one through T cell receptor mediated by Ag and the other from costimulatory molecules (Matzinger and Fuchs 1996). The activation signals for Th cells differ among naive and effector-stage Th cells (Croft 1994). Effector-stage Th cells require fewer stimulatory signals, and when strong signals are provided through T cell Ag receptors by a large dose of Ag, effector-stage Th cells may not require many additional signals through costimulatory molecules. Polynucleotide immunomodulation was not observed with optimal Ag challenges in Th1 and Th2 clones. Thus we speculate that polynucleotides may affect Th cell action by modulating signals of costimulatory molecules.

The effects of polynucleotides supplemented to the cultures were evident with the use of in vivo Ag-primed spleen cells but not with unprimed spleen cells in the assays of Th clone-mediated Ab production and cytokine production. In primed spleen cells, the numbers of activated APC and Ag-specific B cells were higher than in unprimed spleen cells (Bradley et al. 1993). Consequently, primed spleen cells likely stimulate Th clones more effectively due to the presence of larger numbers of activated APC than unprimed spleen cells. They also likely produce a higher number of Ab-secreting cells in the ELISPOT assay secondary to elevated numbers of Ag-specific B cells. As a consequence the effects of polynucleotides may have been more dramatic with primed spleen cells than with unprimed spleen cells. In preliminary studies, we examined numbers of macrophages, B cells, and CD4+ and CD8+ T cells following in vivo Ag challenges. The frequency of B cells and CD4+ T cells increased by 5–10% in this system but the differences were not statistically significant (unpublished observations). We now plan to investigate changes in activation marker expression for B cells and macrophages in response to in vivo Ag challenge (cytochrome C and rabbit β-globulin).

In contrast to the suppressive action of polynucleotides on Th1 clone-mediated Ab production and IL-5 production by Th2 cells, polynucleotides augmented IFNγ production by Th1 cells. This enhancing action of polynucleotides may be attributed to IFNγ production induced by double stranded (ds) RNA contained in the yeast RNA preparations used as the source of polynucleotides (Hubbell et al. 1991, Mémét et al. 1991, Tiwari et al. 1987). However, polynucleotides enhanced IFNγ production with suboptimal but not optimal Ag challenges and did not augment IFNγ production in the absence of Ag. The amount of dsRNA used by others ranged 0.05 to 0.2 g/l (Bourgeade et al. 1993, Decker 1992, Hubbell et al. 1991, Mémét et al. 1991, Tiwari et al. 1987). Such large amounts of dsRNA were unlikely to be provided in the RNA preparations used here, since the highest dose of RNA employed in this study was 0.1 g/l, and the RNA was already degraded to <1,000 nucleotide lengths of predominantly single stranded polynucleotides (Jyonouchi et al. 1992). Therefore, enhancing actions of polynucleotides on IFNγ production by Th1 cells are unlikely to be attributed to the actions of dsRNA. It may be that polynucleotides enhance IFNγ production by Th1 cells by modulating the processes of Ag-mediated Th cell activation; further studies are needed to prove this assumption. IFNγ suppresses humoral immune responses (Paul and Seder 1994). Enhancing actions of polynucleotides on IFNγ production may partly account for the decrease in Th1 clone-mediated Ab production with suboptimal Ag challenges.

In general, most pathogens induce mixed Th1 and Th2 responses. When Ag concentrations are suboptimal, polynucleotides released from the injured tissue may shift local immune responses to Th1 dominant cellular immune responses by enhancing IFNγ production and suppressing IL-5 production. Th1 responses are generally more effective in removing intracellular pathogens such as bacteria and viruses (Biron
1994, Garside and Mowat 1995). It will be interesting to examine the local immune responses in animals challenged with Ag or pathogens.

In summary, polynucleotides neither augmented nor suppressed functions of effector-stage Th cells nonspecifically. Our results demonstrate the complex actions of polynucleotides on Th cell functions, depending on the stage of Th cell activation. Thus they are unlikely to induce hyperactive, uncontrolled immune responses.

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LITERATURE CITED


