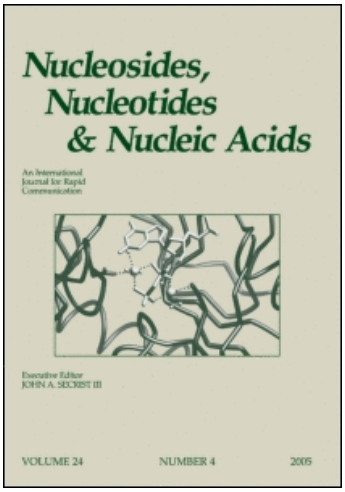

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Activation of Spleen Lymphocytes by Plasmid DNA

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ACTIVATION OF SPLEEN LYMPHOCYTES BY PLASMID DNA.

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ABSTRACT. It was shown that plasmid pUC19 DNA stimulates *in vitro* proliferation of CBA mouse splenocytes in a dose-dependent manner. Stimulation effect of the plasmid DNA is additive with Con A or LPS, synergistic with PMA and is inhibited by nonimmunogenic phosphodiester oligonucleotides and Fab fragments of antimouse Ig antibodies. These data and the data of affinity labelling of ODN-binding proteins indicate that immunoglobulin receptors are involved in DNA-induced lymphocyte activation.

Bacterial DNA and certain oligonucleotides (ODNs) were shown to stimulate immune system and cause unspecific antitumor and antivirus effects [1,2]. These compounds induce T, B and natural killer cells to secrete several cytokines, increase splenic B cells number, class II major histocompatibility complex expression on B cells and immunoglobulin synthesis by splenocites *in vivo* and *in vitro* [2,3].

We investigated here the stimulatory effect of plasmid pUC19 on splenocyte proliferation *in vitro*. pUC19 DNA was reported recently to have an adjuvant effect in gene-vaccinated animals, as well as in animals immunized with protein antigens[3]. We have shown that plasmid pUC19 DNA stimulates *in vitro* proliferation of CBA mouse splenocytes in a dose-dependent manner. Simultaneous treatment of the cells with the plasmid DNA and Con A or LPS produced an additive effect, while PMA acted synergistically with DNA. Nonimmunogenic phosphodiester oligonucleotides caused dose-dependent suppression of the DNA-induced lymphocyte proliferation. Monovalent Fab fragments of rabbit antimouse Ig antibodies (RAMIg) significantly inhibited plasmid

TABLE 1. Inhibition effect of anti-Ig antibodies and their Fab fragments on plasmid DNA-induced spleen lymphocyte proliferation. Mitogen and 50 μg/ml anti-Ig antibodies or Fab fragments were added to cell suspension simultaneously. The results are the mean ± SD of triplicate cultures and are representative of three separate experiments.

Mitogens, concentration		[^{3}H]Thymidine incorporation, cpm·10^3		
			+ anti-Ig	+ Fab
None		2.6 ±0.2	1.9 ±0.2	2.0 ±0.1
DNA,	10 μg/ml	51.5 ±7.2	16.5 ±1.3	26.0 ±3.2
DNA,	1 μg/ml	41.0 ±4.5	9.0 ±1.1	11.0 ±1.4
DNA,	0.1 μg/ml	15.0 ±1.4	4.5 ±0.9	2.8 ±0.4
LPS,	2.5 μg/ml	42.0 ±5.1	20.0 ±3.5	35.0 ±4.2

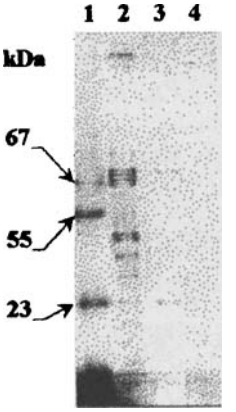

FIG. 1. Isolation of cellular IgM by immunoprecipitation with RAMIg antibodies from membrane-cytosole fractions (MCF) of CBA mouse spleen lymphocytes. Spleen lymphocytes (10^6) were incubated with [^{32}P]CIRp(N)$_{16}$. Total MCF (lane 2) and immunoprecipitates of MCF with RAMIg antibodies, collected on protein A-Sepharose beads (lane 3) were analysed by 10-20% SDS PAGE, followed by autoradiography. Control of nonspecific binding was made with normal rabbit IgG instead of RAMIg (lane 4). Molecular weight markers: BSA and mouse myeloma IgG (lane 1), modified with [^{32}P]CIRp(N)$_{16}$.

DNA-induced polyclonal lymphocyte activation suggesting the involvement of Ig receptors in this process. In experiments with alkylating 4-[(N-2-chloroethyl-N-methyl)amino]benzylamine (ClR-) linked ODNs we have found that high molecular weight DNA prevents the modification of ODN- binding proteins. These data indicate that DNA and ODNs interact with the same splenocyte membrane proteins. Affinity modification of

lymphocytes membrane-cytosole proteins with a [^{32}P]-labeled ClR-ODN resulted in labeling of 67-82 and 23 kDa polypeptides corresponding to IgD and IgM heavy and light chains respectively. The immunoglobulin nature of the 82 and 23 kDa oligonucleotide-binding polypeptides was confirmed by immunoprecipitation with RAMIg antibodies. These data indicate that immunoglobulin receptors are involved in DNA-induced lymphocyte activation.

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