

Interleukin 4 Induces Changes in the Chromatin Structure of the $\gamma 1$ Switch Region in Resting B Cells Before Switch Recombination

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Summary

Interleukin 4 (IL-4) can induce the expression of IgG1 in sIgG⁻ murine B cells stimulated with mitogens or through a cognate interaction with T helper (Th) cells. We have investigated the molecular basis for the IL-4-induced switch to IgG1 in lipopolysaccharide (LPS)-stimulated murine B cells and have previously shown that IL-4 induces transcription of the $\gamma 1$ switch region before switch recombination. We now demonstrate that IL-4 induces a DNase I hypersensitive site at the 5' end of the $\gamma 1$ switch region in resting B cells. LPS is not required, but it enhances induction. Hence, the interaction of IL-4 with its receptor results in increased accessibility of the $\gamma 1$ switch region. The more open chromatin structure and increased transcriptional activity may be important in the selection of this region for switch recombination.

T helper cells and Th cell-derived lymphokines play an important role in the regulation of isotype switching and expression. In particular, IL-4 induces switching to IgG1 (1, 2) and IgE (3) in mitogen-activated B cells in vitro. Several reports have suggested that mitogens and some lymphokines may direct isotype switching by increasing the accessibility of specific switch regions to switch recombinase(s) (4-6). In support of this hypothesis, we and others have demonstrated transcriptional activation of the $\gamma 1$ (6-8) and ϵ (9) switch regions by IL-4 before switch recombination in murine splenic B cells. In addition, Schmitz and Radbruch (10) have recently shown that the combination of LPS and IL-4 can induce the appearance of a single DNase I hypersensitive site upstream of the $\gamma 1$ switch region before switch recombination. In the present study we report the induction of two distinct DNase I hypersensitive sites at the 5' end of S $\gamma 1$ by LPS plus IL-4. We further demonstrate that IL-4 alone can induce the stronger of these two hypersensitive sites in resting B cells without co-stimulation with LPS. Thus, IL-4 can deliver signal(s) that open the $\gamma 1$ switch region and perhaps make it more accessible to the switch recombination machinery of the cell.

Materials and Methods

Animals. Specific pathogen-free female BALB/c mice were obtained from Simonsen Laboratories (Gilroy, CA) and were housed in laminar-flow animal isolation hoods. All animals were used at 8-16 wk of age.

Preparation and Culture of Splenic B Cells. Small, resting B cells

(density >1.085 g/ml) were prepared by Percoll density gradient fractionation and cultured as previously described (7). LPS (*Salmonella typhosa* 0901, Difco Laboratories, Detroit, MI) was used at a concentration of 20 μ g/ml. rIL-4 was purified as described previously (7) and was used at a final concentration of 200-500 units/ml.

Preparation of Nuclei and Digestion with DNase I. Nuclei were purified by a modification of the method of Wu (11). $1-2 \times 10^8$ cells were harvested by centrifugation (400 g), washed once in ice-cold PBS, and once in nuclear isolation buffer (NIB) (60 mM KCl, 15 mM NaCl, 5 mM MgCl₂, 0.1 mM EGTA, 15 mM Tris-HCl [pH 7.4], 0.5 mM dithiothreitol, 0.1 mM PMSF, 0.3 M sucrose). Cells (5×10^7 /ml) were disrupted in NIB containing 0.1-0.5% NP-40 for 5 min on ice. The nuclei were pelleted through a cushion of NIB containing 1.7 M sucrose and resuspended at $3-5 \times 10^7$ nuclei/ml in NIB supplemented with 5% glycerol. Aliquots of nuclei were digested for 5 min at 25°C with increasing concentrations of DNase I (Boehringer Mannheim Biochemicals, Indianapolis, IN) administered in 0.1 \times volume of 5 mM CaCl₂, 1 mM MgCl₂. The digestions were stopped by the addition of an equal volume of 20 mM EDTA, 1% SDS. Samples were digested for 16 h at 50°C with proteinase K (100 μ g/ml) and the DNA was purified by repeated phenol extraction followed by ethanol precipitation.

Southern Blotting Analysis. DNA samples were digested to completion with EcoRI, electrophoresed on agarose gels, and blotted to nylon membranes (Zetaprobe; Bio-Rad Laboratories, Fullerton, CA) according to the alkaline blotting procedure of Reed and Mann (12). Blots were hybridized to purified restriction fragments labeled with ³²P to a specific activity of $1-2 \times 10^9$ cpm/ μ g by random priming (13). Hybridization and washing conditions were according to Church and Gilbert (14) as modified in the Zetaprobe hybridization protocol (Bio-Rad Laboratories). Blots were exposed to x-ray film with an intensifying screen at -70°C.

Results and Discussion

IL-4-mediated Induction of DNase I Hypersensitive Sites in the $\gamma 1$ Switch Region of LPS-stimulated B Cells. IL-4 induces LPS-stimulated sIgG⁻ B cells to switch to the expression of IgG1 (1, 2) and is required during the first 48 h of culture (15). We have shown previously that IL-4 induces the expression of germline transcripts from the unrearranged $\gamma 1$ locus in LPS-stimulated B cells within 4 h of culture (7). In the present studies, we determined whether IL-4 could induce changes in the chromatin structure of the S γ 1 region in small, resting, splenic B cells co-stimulated with LPS. Nuclei were prepared from cells cultured for 12 h with LPS (20 μ g/ml) or LPS and rIL-4 (450 U/ml) and were treated with increasing concentrations of DNase I. The purified DNA was digested to completion with EcoRI and analyzed on Southern blots. The blots were hybridized with probes specific for either end of the 16-kb EcoRI restriction fragment containing the S γ 1 region (Fig. 1 A). This indirect labeling procedure (11) allows detection and mapping of any DNase I hypersensitive sites within the 16-kb EcoRI fragment based on the size of the subfragments generated by DNase I digestion.

Hybridization with the 3' S γ 1 probe (Fig. 1 A) revealed the expected 16-kb fragment, which diminished in intensity in both sets of samples with increasing concentrations of DNase I. In DNA prepared from B cells cultured with LPS and rIL-4, a prominent 10.5-kb subfragment and a weaker 8.0-kb subfragment were present after digestion with DNase I, indicating the presence of DNase I hypersensitive sites \sim 10.5 kb and 8.0 kb upstream of the 3' EcoRI site. A comparison of the migration of the DNA fragments generated by DNase I and the fragments generated by partial digestion of genomic DNA with BglII followed by complete digestion with EcoRI (Fig. 1 B, lane M) demonstrates that the major DNase I hypersensitive site is very close to (\pm 200 bp) the 3' BglII restriction site at the 5' end of the $\gamma 1$ switch region as shown in Fig. 1 A. In separate experiments (data not shown), hybridization of EcoRI digests with a 5' probe mapped the DNase I hypersensitive sites relative to the 5' EcoRI site and confirmed the results with the 3' probe.

As a positive control of DNase I digestion of the chro-

matin from B cells cultured with LPS alone, all blots were rehybridized with a probe specific for the $\gamma 2b$ switch region (Fig. 1 C), a region previously shown to be transcriptionally active in LPS-stimulated B cells (5) and which might also be expected to contain DNase I hypersensitive sites. This analysis demonstrated a strong hypersensitive site at the 5' end of S γ 2b in B cells cultured with LPS alone as well as in cells cultured with LPS and rIL-4 (Fig. 1 C). The S γ 2b hypersensitive site was easily detected in B cells cultured with LPS alone at DNase I concentrations at which no DNase I hypersensitive sites could be detected in the $\gamma 1$ switch region of the same cells. Furthermore, only minor differences were observed among the DNA preparations from cells cultured with LPS or LPS and rIL-4 with respect to the intensities of the S γ 2b subfragments or the concentration of DNase I required for their detection. Therefore, the failure to detect the S γ 1 hypersensitive sites in B cells cultured with LPS alone is not due to the failure of the DNase I to digest the chromatin in these nuclei.

IL-4 Alone Induces the Major DNase I Hypersensitive Site in the $\gamma 1$ Switch Region of Resting B Cells. We have recently shown that IL-4 can induce the synthesis of germline $\gamma 1$ transcripts in resting B cells without co-stimulation with LPS (7). We therefore investigated the possibility that IL-4 could also induce changes in the S γ 1 chromatin structure of resting B cells in the absence of LPS. B cells were cultured for 24 h in medium containing 200 U/ml of rIL-4, LPS alone, or LPS and rIL-4. Nuclei were prepared and the S γ 1 chromatin was analyzed for DNase I hypersensitive sites. As an additional negative control, nuclei from freshly isolated B cells were also analyzed. As shown in Fig. 2, rIL-4 alone induced the same major DNase I hypersensitive site in S γ 1 that was induced by the combination of LPS and rIL-4. This site was consistently induced to a greater extent in cells cultured with LPS and rIL-4, suggesting that LPS may have an enhancing effect as we reported previously for the induction of germline $\gamma 1$ transcripts (7). The 8.0-kb subfragment indicative of the minor hypersensitive site was not consistently induced above background in the cells cultured with IL-4 alone. The hypersensitive sites were absent or only barely detectable in

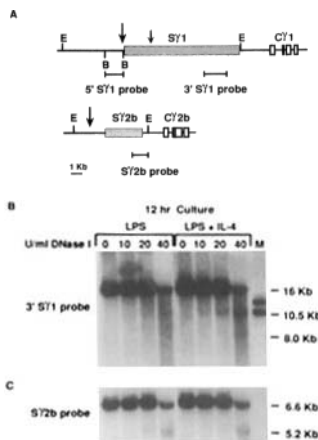


Figure 1. LPS and IL-4 induce a DNase I hypersensitive site near the 5' end of S γ 1 in normal B cells. (A) Restriction maps of the BALB/c $\gamma 1$ and $\gamma 2b$ constant region loci (17, 18). The positions of the switch regions (stippled boxes) and of the constant region exons (open boxes) are indicated as are the positions of relevant restriction sites: E, EcoRI; B, BglII. The BglII and BamHI S γ 1 restriction fragments used as probes in these studies were subcloned from p $\gamma 1$ /EH10.0 (17) (kindly provided by Dr. W. Dunnick, U. Michigan) and are also indicated below the map. The S γ 2b probe was kindly provided by Dr. K. Marcu, SUNY (19). (B) Small, resting B cells were cultured for 12 hours with LPS alone (20 μ g/ml) or with LPS and rIL-4 (450 U/ml). Nuclei were prepared and were treated with the indicated concentrations of DNase I as described in Materials and Methods. DNA purified from the B cell nuclei was digested to completion with EcoRI, electrophoresed (2 μ g/lane) on a 0.8% agarose gel, blotted to a nylon membrane, and hybridized to the 3' probe shown in A. The sizes in kilobases indicated to the right of the blot refer to the sizes of the germline EcoRI fragment (16 kb) and the subfragments (10.5 and 8.0 kb) generated by DNase I digestion. For markers (lane M), DNA from undigested B cell nuclei was partially digested with BglII, completely digested with EcoRI, and analyzed in parallel. In addition, a 1-kb ladder (Bethesda Research Laboratories) was included as M_r markers on all gels (data not shown). (C) The blot shown in B was stripped and rehybridized with the S γ 2b probe shown in A.

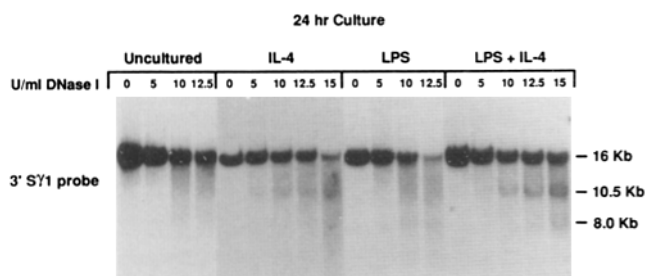


Figure 2. IL-4 alone induces alterations in the $S\gamma 1$ chromatin of resting B cells. Nuclei from uncultured small, resting B cells and from B cells cultured for 24 h with rIL-4 alone (200 U/ml), LPS alone, or LPS and rIL-4 were analyzed for DNase I hypersensitive sites in $S\gamma 1$ as described in Fig. 1.

uncultured B cells and in B cells cultured with LPS alone, although reprobing with the $S\gamma 2b$ probe demonstrated equivalent digestion of these nuclei by DNase I (data not shown).

The data presented in this report demonstrate that a T cell-derived lymphokine can provide the signal(s) necessary to induce structural changes in switch region chromatin before switch recombination in B cells. Our results confirm and extend the recent report by Schmitz and Radbruch (10) that the combination of LPS and IL-4 can induce a DNase I hypersensitive site upstream of $S\gamma 1$. However, we also detect at least one additional, minor DNase I hypersensitive site induced by LPS plus IL-4. Moreover, we demonstrate that IL-4 alone can induce the major DNase I hypersensitive site and that LPS enhances the induction. Interestingly, IL-4 had no effect on a DNase I hypersensitive site in the $\gamma 2b$ switch region, although it inhibits transcription of this region in

normal spleen cells stimulated with LPS (5). The IL-4 mediated induction of DNase I hypersensitive sites upstream of and within $S\gamma 1$ in B cells reflects an increase in the accessibility of the $S\gamma 1$ chromatin to DNase I and presumably to other enzymes, such as RNA polymerases and switch recombinase(s). Such increased accessibility has been associated with increased transcriptional activity in many other systems (16), and we and others have shown that IL-4 induces transcripts from the unrearranged $\gamma 1$ (7, 8) and ϵ switch regions (6, 9) before switch recombination to these regions. Preliminary experiments indicate that the germline $\gamma 1$ transcripts induced by IL-4 initiate very near the most prominent DNase I hypersensitive site and just 5' of the 3' Bgl II site (Berton, M.T., and E.S. Vitetta, unpublished data).

Our observations support a model of regulated isotype switching in which the selection of a particular isotype for expression (other than μ or δ) is determined by the accessibility of the appropriate switch region to a switch recombinase (4-6). In the context of this model, IL-4 presumably induces the expression or activation of DNA-binding proteins that recognize promoter elements or enhancer-like sequences upstream of $S\gamma 1$ and $S\epsilon$. The binding of these proteins to these elements would make these regions more accessible to RNA polymerases. The resultant transcription may then stabilize the more open chromatin structure and set the stage for switch recombination when additional signals are received. Although the role of LPS is not known, it clearly can provide the signals required to complete the process either by enhancing the transcriptional signal delivered by IL-4 (7) and/or by activating the switch recombination machinery by inducing cell proliferation.

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