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Heparin alters the expression of different forms of immunoglobulin μ heavy chains and their associated proteins by pre-B cell lines and normal Ly-1 (CD5+) B cells

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Abstract

Studies presented here show that heparin alters immunoglobulin expression by murine pre-B cell lines and normal Ly-1 (CD5+) B cells. Previous studies have shown that pre-B cell lines 70Z/3 and NFS-5.3 express μ heavy chains in the cytoplasm and a small amount on the cell surface. Both these cytoplasmic and surface μ are disulfide-linked to ω (λ_5) surrogate light chains and are noncovalently associated with ι ($V_{pre\text{-}B}$) variable region-like proteins. We show that culturing 70Z/3 with heparin reduces the amount of the membrane-form μ (μm) on the cell surface. Culturing NFS-5.3 with heparin similarly decreases the membrane-form μ; however, it increases the surface level of a pentameric μ molecule containing secreted-form μ (μs) heavy chains, disulfide-linked ω (λ_5) chains, and noncovalently associated proteins. Culturing peritoneal B cells with heparin also increases the production of the secreted-form μs, detectable in this case by the secretion of classical pentameric IgM. Similarly, injecting heparin intraperitoneally increases IgM secretion by peritoneal Ly-1 B cells. Thus heparin could influence pre-B cell and B cell differentiation and function.

Introduction

Pre-B cells have functionally rearranged one of the μ heavy chain genes (1). Cell lines derived from pre-B cells transcribe the μ gene to yield two kinds of mRNAs, one coding for the membrane-form of the μ heavy chain and the other coding for the secreted-form (2–4). Both of these mRNAs are translated into μ heavy chains that are found in the cytoplasm (5–8). A small amount of the μ produced reaches the cell surface, little is secreted (9,10). Recent findings show that these μ chains, either in the cytoplasm or on the cell surface, are linked via disulfide bonds to ω (λ_5) surrogate light chains and noncovalently associated with ι ($V_{pre\text{-}B}$) variable region-like proteins (11–17).

Rearrangement and expression of either the κ or λ light chain gene in pre-B cells results in the formation of mature B cells. These cells mainly produce the membrane form of IgM and IgD and express these immunoglobulins on the cell surface in association with characteristic sets of glycoproteins (18–22). Stimulation of mature B cells with antigens and/or lymphokines under appropriate conditions triggers a shift towards the production of mRNA for secreted-form μ and the secretion of large amounts of IgM antibodies (23).

A variety of lymphokines and mitogens have been shown to alter immunoglobulin expression by mature B cells (24,25). For example, interleukin 5 (IL-5) induces IgM secretion by normal Ly-1 B cells and Ly-1 B leukemic cells (BCL-1) (26,27), whereas IL-4 induces IgG1 or IgE secretion by lipopolysaccharide (LPS)-activated B cells (28). In pre-B cell lines, dextran sulfate has been shown to increase the expression of surface μ in the absence of light chain (9). IL-1, γ-interferon (γ-IFN), and LPS have been shown to induce the expression of Ig light chains and the consequent IgM expression on the cell surface (10,29,30).

Studies presented here show that heparin, a widely distributed and highly negatively charged proteoglycan produced by a variety of cell types (31–33), modulates immunoglobulin expression by B cells at different stages of differentiation. It stimulates IgM secretion by normal peritoneal Ly-1 (CD5+) B cells. In addition, in a pre-B cell line NFS-5.3, it increases the

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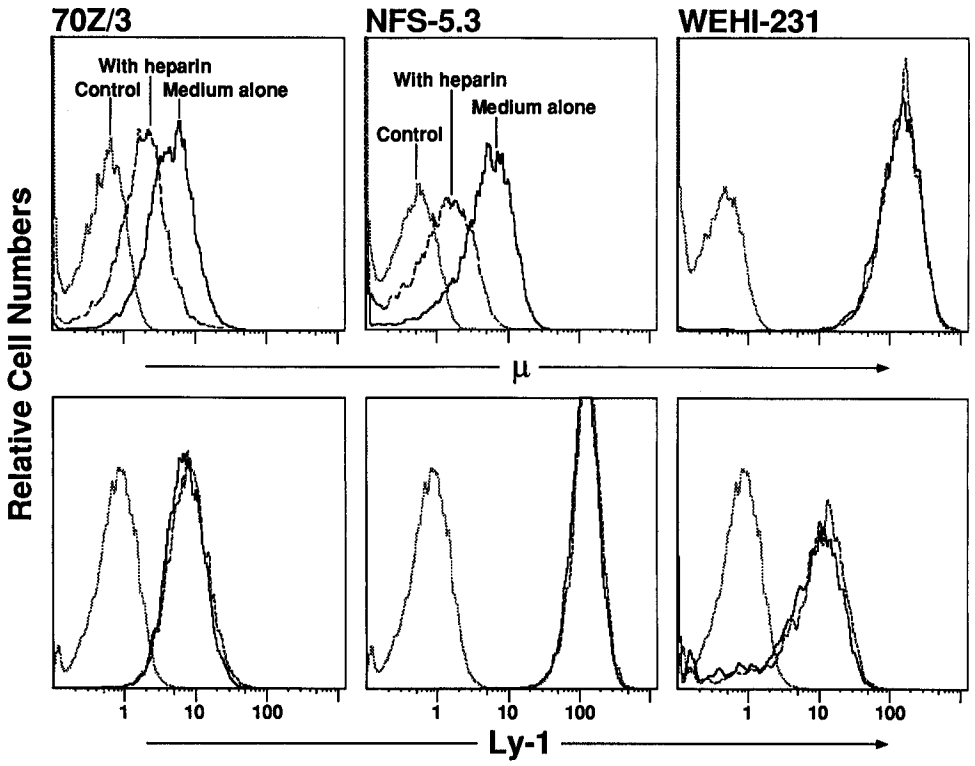

Fig. 1. Heparin induces a partial μ reduction on pre-B cell lines. 70Z/3 and NFS-5.3 cells were cultured for 18 – 24 h in RPMI medium or in RPMI medium with 100 μg/ml heparin. Cells were washed, stained with APC-conjugated anti-μ, anti-Ly-1, or APC-conjugated irrelevant isotype-matched control antibodies, and analyzed by FACS. 10,000 live cells were collected for each sample to obtain the histogram. Medium alone, anti-μ, or anti-Ly-1 stains of cells cultured in RPMI; with heparin, anti-μ or anti-Ly-1 stains of cells cultured in RPMI in the presence of 100 μg/ml heparin; control, stains of irrelevant isotype-matched control antibodies (GK1.5: rat anti-mouse L3T4; 53-6.7: rat anti-mouse Lyt-2) of cells cultured in RPMI. Culturing pre-B cell lines Ig6.3 and Ig6.11 in the presence of heparin also induces a partial reduction of surface μ (data not shown). Heparin apparently does not alter the rate of cell proliferation nor causes cell death in the culture.

surface expression of an immunoglobulin pentamer containing secreted-form μs heavy chains, covalently linked ω (λ_5) surrogate light chains, and noncovalently associated proteins. Finally, it decreases the expression of membrane-form μm which exists as a four-chain molecule ($\mu m_2\omega_2$) on this cell line and on another commonly studied pre-B cell line, 70Z/3. These findings suggest that heparin is potentially important in regulating aspects of B cell differentiation and function.

Methods

Reagents and cell lines

Heparin was from Upjohn (NDC 0009-0268-01, Kalamazoo, MI) and was precipitated in 0.3 M sodium acetate by 70% ethanol three times before use. Heparin sulfate, chondroitin-4-sulfate, and chondroitin-6-sulfate were gifts from Dr Merton R. Bernfield and were further purified in the same way.

The following monoclonal antibodies were used: 331.1, a rat IgG2b anti-mouse μ heavy chain, used for staining and immunoprecipitation; MAR 18.5, a mouse IgG2a anti-rat κ light chain, used as a second step antibody for 331.1 immunoprecipitation; 53-7.8, a rat IgG2a anti-mouse Ly-1, used for staining; rat IgG1 anti-mouse IgE (Rm-E-02 P, Pharmingen, San Diego, CA), used for sensitizing mast cells; and rat IgG1 anti-dinitrophenol (DNP), used as an isotype-matched control.

Cell lines used include pre-B cell lines 70Z/3, NFS-5.3, HAFTL-1, Ig6.3, and Ig6.11 (a gift from Dr M. Taniguchi); and B cell lines WEHI-231, AJ9, and NFS-1.0. Cells are cultured in RPMI 1640 with 10% fetal calf serum (FCS), 1 mM glutamine, 2 mM sodium pyruvate, 50 U/ml penicillin, 50 μg/ml streptomycin, and 50 μM 2-mercaptoethanol. For heparin treatment, cells were cultured in the same medium in the presence of 100 μg/ml heparin for 18 – 24 h. For LPS (CAT #3124-25-6, DIFCO, Detroit, MI) induction, cells are cultured in the same medium in the presence of 10 μg/ml LPS for 18 – 24 h.

Fluorescence-activated cell sorter (FACS) analysis

Cells from different treatments were washed, stained and analyzed by FACS. Anti-μ monoclonal antibody 331.1 is an allophycocyanin (APC) conjugate. The reagent is very bright and good for detecting low levels of surface μ. Other conjugated monoclonal antibodies were kindly provided by Dr Alan M. Stall. Dead cells were excluded by propidium iodine staining.

Immunoprecipitation and gel analysis

Surface labeling and immunoprecipitation were carried out as described (18,34). Briefly, cultured cells were labeled with ^{125}I

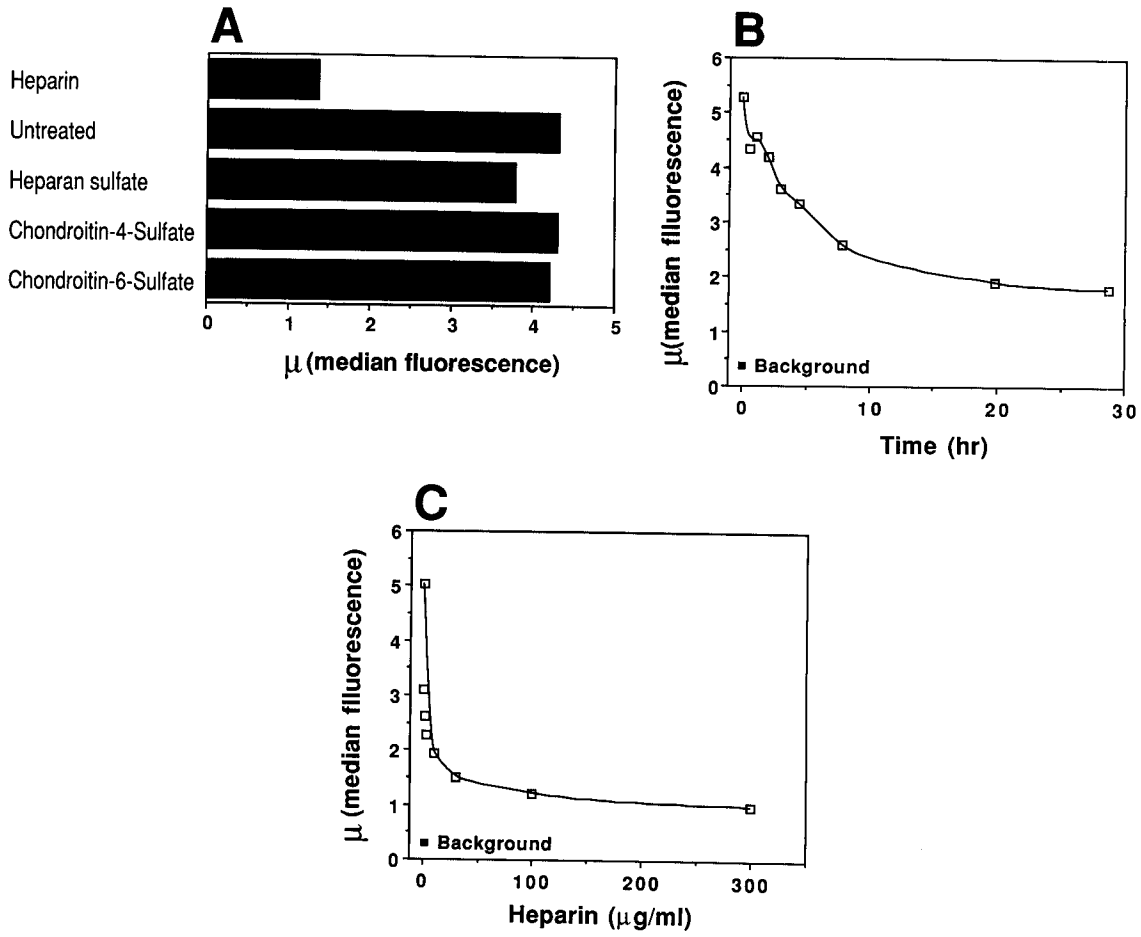

Fig. 2. (A) Comparison of the abilities of proteoglycans in inducing surface μ reduction on pre-B cells. NFS-5.3 cells were cultured in RPMI in the presence of 100 μg/ml heparin, heparan sulfate, chondroitin-4-sulfate or chondroitin-6-sulfate for 24 h. Cells were washed, stained with anti-μ, and analyzed by FACS. Median μ fluorescences are compared. (B) Kinetics of surface μ reduction by heparin treatment. NFS-5.3 cells were cultured in the presence of 100 μg/ml heparin. Cell aliquots were removed from the culture at different times and analyzed for surface μ level by staining and FACS. Median μ fluorescences are used for the plot. Background indicates median fluorescence level stained with an irrelevant isotype-matched control antibody (GK1.5). (C) A dose – response curve of surface μ reduction by heparin treatment. NFS-5.3 cells were cultured in the presence of different concentrations of heparin for 18 h and then analyzed for surface μ level by staining and FACS. Median μ fluorescences are used for the plot.

by the glucose oxidase/lactoperoxidase method and lysed in NP-40 or digitonin lysis buffer. Cell lysates were precleared with Sansorbin (Calbiochem, San Diego, CA) and then precipitated with anti-μ (331.1) monoclonal antibody that had already been absorbed to Pansorbin (Calbiochem). Immunoprecipitates were analyzed by SDS – PAGE and by diagonal gels (nonreduced – reduced two-dimensional gel electrophoresis). Alternatively, immunoprecipitates were treated with *N*-glycanase™ (Genzyme, Boston, MA) before SDS – PAGE analysis. Prestained protein standards (Bio-Rad, Richmond, CA) were used for relative molecular mass determinations. Gels were dried and autoradiographed with Kodak XR films with intensifying screens at −70°C.

RNA isolation and Northern analysis

Cytoplasmic RNA was isolated from NFS-5.3 and 70Z/3 cells (35). This total RNA was fractionated on a 1.0% agarose gel, transferred to a nitrocellulose filter and hybridized sequentially with DNA probes derived from Cμ3, 4 exon, secretory exon, and transmembrane exon after removal of the previously hybridized probe.

Bromelain plaquing assay

BALB/c and CBA/Ca mice at age of 3 – 5 months (~30 g) were injected intraperitoneally with 1 ml deficient RPMI (normal RPMI 1640 without biotin, riboflavin, phenol red, glutamine, sodium pyruvate, and FCS) containing 300 μg heparin or 30 μg monoclonal anti-IgE antibody. As controls, littermates were injected intraperitoneally with 1 ml medium or 1 ml medium containing 30 μg isotype-matched control antibody. Peritoneal cells were collected for bromelain plaquing assay 24 h after injection. For *in vitro* experiment, peritoneal cells were removed, cultured for 24 h in RPMI medium alone, with 100 μg/ml heparin, with 5 μg/ml anti-IgE antibody, or with 5 μg/ml isotype-matched control antibody, and followed by bromelain plaquing assay. Bromelain-treated mouse erythrocytes were prepared according to Cunningham and Szenberg (36). Plaque-forming cells (PFC)

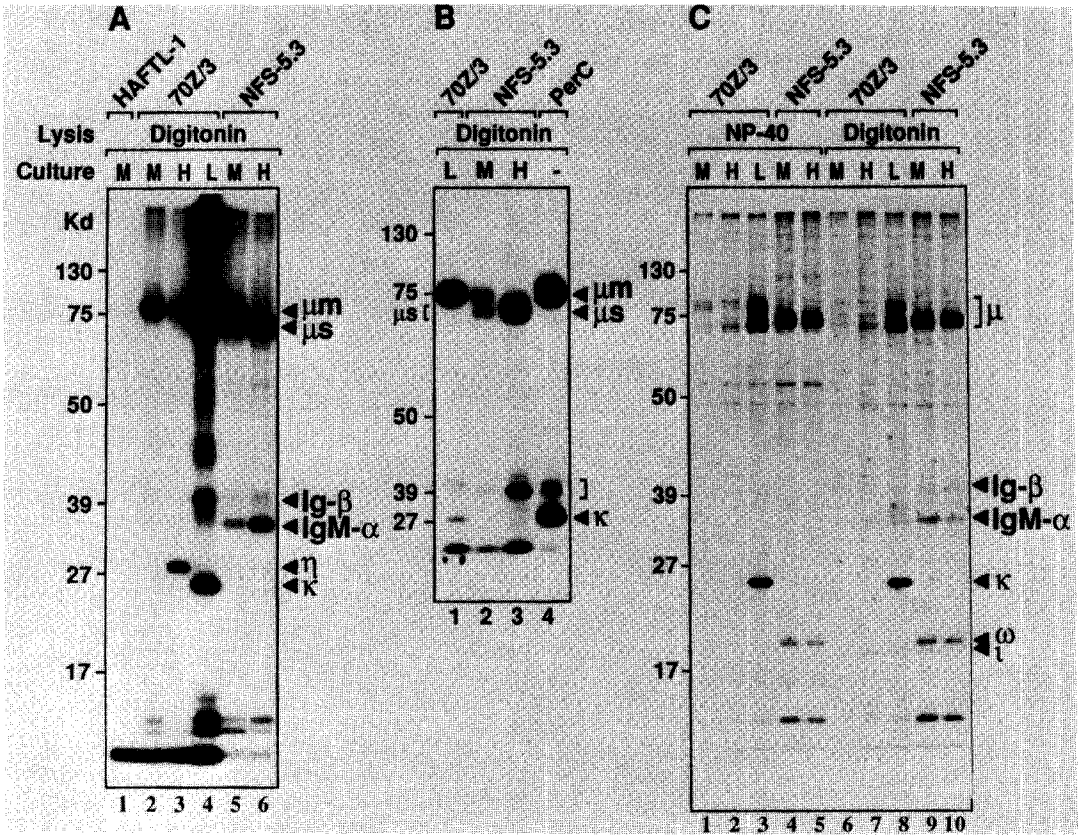

Fig. 3. SDS – PAGE analyses of anti-μ precipitates from pre-B cell lines and normal murine peritoneal B cells. M, medium alone; H, with 100 μg/ml heparin; and L, with 10 μg/ml LPS. PerC, murine peritoneal cells. IgM-α, Ig-β, ω and ι are associated proteins. (A) The same number of cells from various cultures were labeled with ^{125}I by lactoperoxidase and lysed with digitonin. Anti-μ precipitates from the same number of cells were analyzed by 12% SDS – PAGE under reducing conditions. HAFTL-1 is a pre-B cell line with heavy chain gene rearrangement but does not make any μ heavy chain protein. (B) The same immunoprecipitates were analyzed by 5 – 12% gradient SDS – PAGE under reducing conditions. The position of the secreted-form μs heavy chain is determined by Coomassie blue staining of reduced pentameric IgM (CBPC 112) in the same SDS – PAGE prior to autoradiography. (C) The same number of cells from various cultures were biosynthetically labeled with [^{35}S]methionine for 4 h and lysed with NP-40 or digitonin. Anti-μ precipitates from the same number of cells were analyzed by 12% SDS – PAGE under reducing conditions. The heterogeneity of biosynthetically labeled μ chains is probably due to an incomplete post-translational processing.

were developed by using rabbit complement in Cunningham chambers (36).

Results

Heparin induces a partial reduction of surface μ on pre-B cell lines

Pre-B cell lines 70Z/3 and NFS-5.3 have productively rearranged both μ and κ genes. However, they express only μ heavy chains which are disulfide-linked to ω (λ_5) chains and noncovalently associated with ι ($V_{pre\text{-}B}$) chains (11,12,14 – 17). These complexes are present in small amounts on the cell surface and the μ heavy chains are clearly detectable by FACS analysis (Fig. 1) (9,10). Culturing 70Z/3 and NFS-5.3 in the presence of heparin, however, substantially decreases the amount of surface μ detectable, while the expression of other surface markers including Ly-1 (Fig. 1), BLA-1, BLA-2, BP-1, and B220 (data not shown) is not affected. Furthermore, the reduction is induced specifically by heparin but not by other structurally related proteoglycans, such as heparin sulfate, chondroitin-4-sulfate, and chondroitin-6-sulfate (Fig. 2A).

The reduction of surface μ is not a simple modulation of surface μ by heparin molecules. Culturing NFS-5.3 with heparin slowly decreases surface μ expression and the kinetics of reduction follows a logarithmic decay curve (Fig. 2B). In contrast, surface μ is completely removed within 15 min by anti-μ modulation. Regeneration of surface μ by NFS-5.3 after culture with heparin requires 14 h, whereas only 4 h are required to fully regenerate surface μ level after anti-μ treatment.

Heparin induces only a partial reduction of surface μ. Culturing 70Z/3 or NFS-5.3 in the presence of 100 μg/ml heparin for 24 h induces a 5-fold reduction of surface μ on all the cells (Fig. 1). This reduction is not significantly induced further by culturing cells with higher concentrations of heparin or with the same concentration of heparin for a longer period of time (Fig. 2B and C). Since heparin does not decrease surface IgM levels on IgM$^+$ B cell lines such as WEHI-231 (Fig. 1), AJ9, and NFS-1.0 and murine spleen B cells (data not shown) the remaining surface μ may be associated with κ light chains instead of ω chains. However, no κ light chains are detected by FACS analyses unless 70Z/3 and NFS-5.3 are stimulated by LPS (data not shown). Alternatively, the remaining surface μ may be different from the

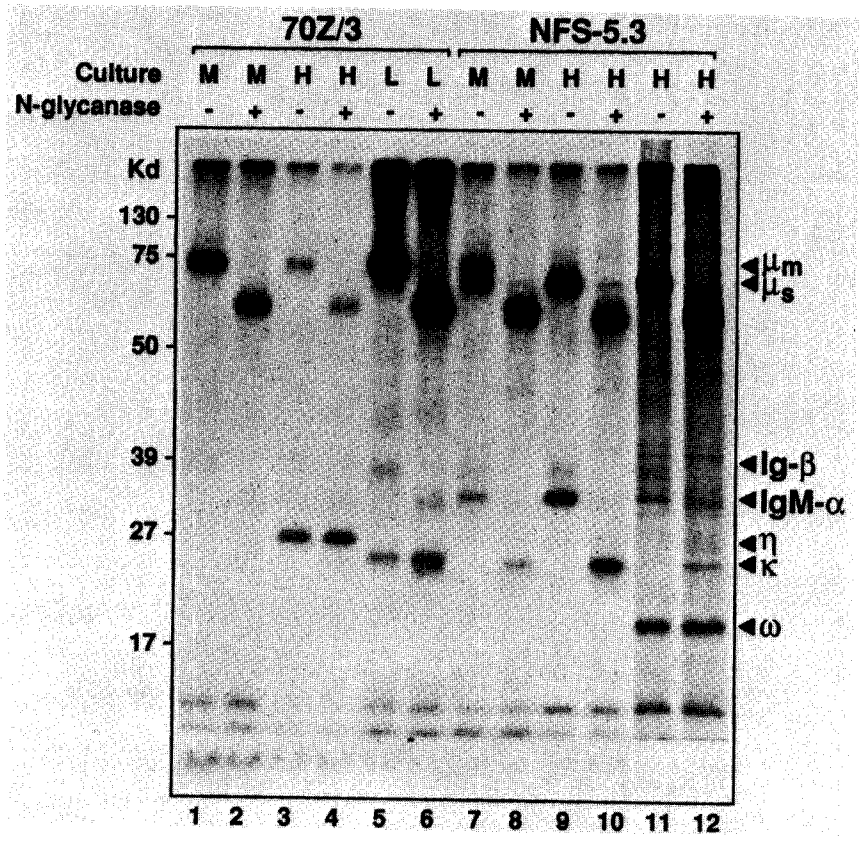

Fig. 4. *N*-glycanase™ treatment and SDS – PAGE analysis of anti-μ precipitates from various culture conditions. The same anti-μ precipitates as in Fig. 3 were treated with *N*-glycanase™ overnight. The mixture was analyzed by 12% reduced SDS – PAGE. M, medium alone; H, with heparin; and L, with LPS. Lanes 1 – 10, ^{125}I-surface iodinated precipitates; lanes 11 and 12, [^{35}S]methionine biosynthetically labeled precipitates.

surface μ lost during culture with heparin but this cannot be distinguished by FACS analyses.

Partial reduction of surface μ on 70Z/3 and NFS-5.3 after culture with heparin is further demonstrated by immunoprecipitation. The same number of cells from different culture conditions were surface-iodinated and lysed with digitonin. Anti-μ precipitates from equal numbers of cells were analyzed by 12% SDS – PAGE under reducing conditions (Fig. 3A). Precipitates from untreated 70Z/3 cells give rise to a band at ~75 kd, which is the same size as the membrane-form μm heavy chain of surface IgM from normal murine peritoneal B cells (Fig. 3B). 70Z/3 cells induced by LPS express a large amount of IgM on the surface as shown by the intense bands of both 75 kd μm heavy chains and 25 kd κ light chains. In contrast, 70Z/3 cells cultured with heparin show a partial reduction in intensity of the 75 kd μm heavy chain band, consistent with findings by FACS analyses. The remaining surface μ on 70Z/3 are identical in size as shown by SDS – PAGE, diagonal gels (data not shown), and deglycosylation treatment (Fig. 4, lanes 2, 4 and 6). As with FACS analyses, no κ light chains are detected by immunoprecipitation unless cells are stimulated by LPS.

Surprisingly, NFS-5.3 cells under normal culture conditions express two different sizes of μ heavy chains on the surface (Fig. 3A, lane 5). The larger one has the same size as the μm heavy chain from surface-labeled 70Z/3 cells and normal murine peritoneal B cells (Fig. 3B). Thus, it is the membrane-form μm. As we will show, the smaller one is most likely the secreted-form μs. Culturing NFS-5.3 with heparin, in contrast, results in a total disappearance of the μm and an increase of the μs (Fig. 3A, lane 6). Thus, heparin specifically decreases the expression of μm on NFS-5.3.

Heparin affects surface μ expression at a post-translational level

The reduction of surface μ expression of pre-B cells by heparin is likely at the post-translational level. The expression level of both μs and μm transcripts is not affected by culturing the cells with heparin (Fig. 5). Neither does heparin inhibit biosynthesis of μ heavy chains (Fig. 3C). After deglycosylation, biosynthetically labeled anti-μ precipitates from heparin-treated NFS-5.3 cells give rise to both 63 and 60 kd bands (Fig. 4, lane 12), indicating that heparin does not affect N-linked glycosylation. In contrast, deglycosylated surface μ from heparin-treated NFS-5.3 cells give rise to a predominant band at 60 kd (Fig. 4, lane 10) while deglycosylated surface μ from untreated NFS-5.3 cells give rise to a predominant band at 63 kd and a faint band at 60 kd (Fig. 4, lane 8). Thus, heparin appears to affect the transportation of μm onto the cell surface.

The remaining μ on NFS-5.3 are likely secreted-form μs

The remaining μ on NFS-5.3 has the same size as the secreted-form μs heavy chain of purified pentameric IgM (Fig. 3B). NFS-5.3, as well as 70Z/3, used in the study has the potential

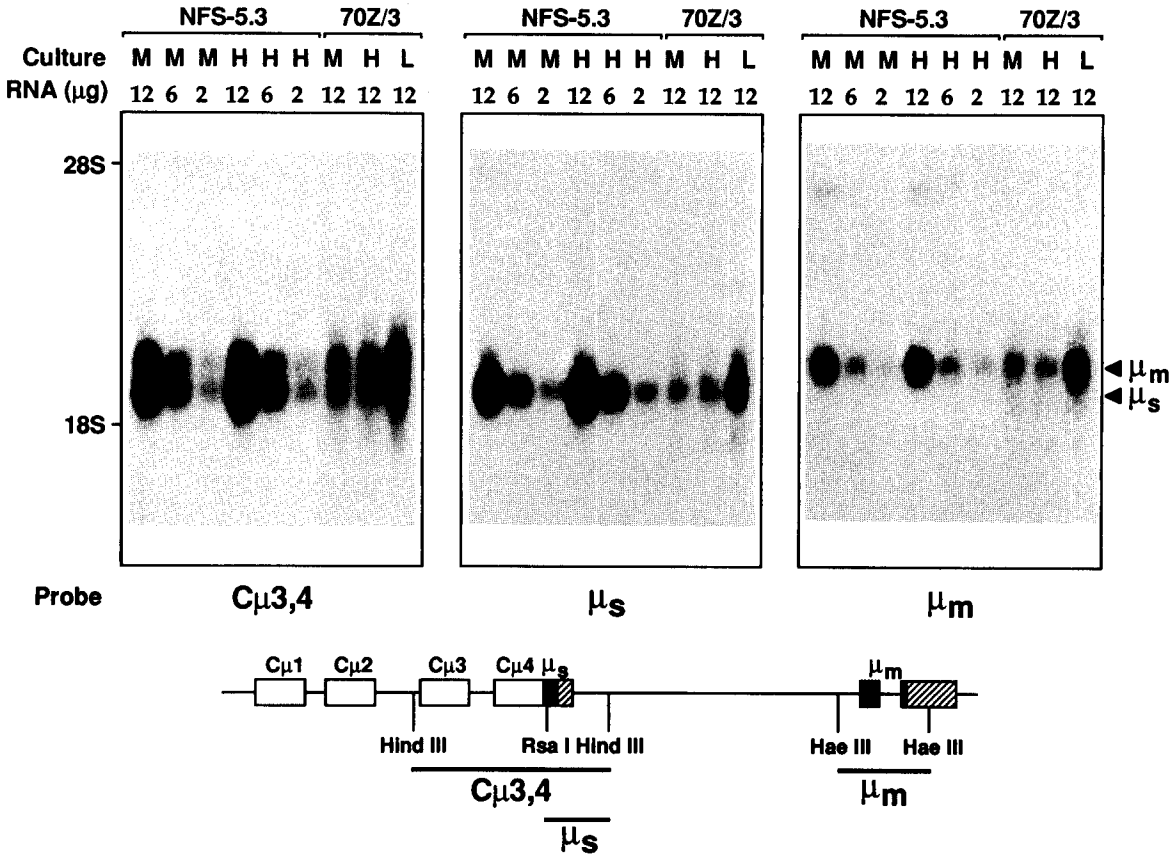

Fig. 5. Northern analyses of μ transcripts from untreated, LPS-induced and heparin-treated 70Z/3 and NFS-5.3 cells. Cytoplasmic RNA was isolated, fractionated in a 1.0% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized sequentially with DNA probes derived from Cμ3, 4 exon (1.2 kb *Hin*dIII – *Hin*dIII fragment), secretory exon (374 bp *Rsa*I – *Hin*dIII fragment) and transmembrane exon (460 bp *Hae*III – *Hae*III fragment) after removal of the previously hybridized probe. M, medium alone; H, with heparin; and L, with LPS. The open, solid and dotted boxes represent Cμ, μs and μm exons respectively. The hatched boxes are 3′ untranslated regions of the secreted-form μs and the membrane-form μm RNA. The smaller (2.4 kb) transcript is the secreted-form μs and the larger (2.7 kb) transcript is the membrane-form μm (3,4,53). 12, 6 and 2 μg RNA from untreated and heparin-treated NFS-5.3 were loaded for titration.

to synthesize μs heavy chains since both cell lines express at least as much μs as μm mRNA transcripts (Fig. 5). 70Z/3 has been shown to synthesize the μs heavy chains (8). In addition, deglycosylated μ from untreated NFS-5.3 give rise to two bands (Fig. 4, lane 8). One is at ~63 kd, the same size as deglycosylated μm from 70Z/3. The other is less in intensity and smaller in size at ~60 kd. Therefore, the size differences are probably not due to differences in N-glycosylation.

The remaining μ on NFS-5.3 exist as pentamers, further suggesting their secreted-form in nature. When analyzed by 2 – 15% gradient SDS – PAGE under nonreducing conditions (Fig. 6), the remaining μ migrate as a single band which have approximately the same size as purified pentameric IgM (CBPC 112). In contrast, surface μ from untreated NFS-5.3 give rise to two bands, one is at about the same size as purified pentameric IgM and the other is slightly smaller than monomeric IgM. Consistently, surface μ from untreated 70Z/3 yields a single band which is slightly smaller than monomeric IgM ($\mu m_2 \varkappa_2$) from LPS-stimulated 70Z/3 and normal murine peritoneal B cells. This small difference in size is because surface μm on these pre-B cell lines are disulfide-linked to ω surrogate light chains which are smaller than $\varkappa$ light chains (20 versus 25 kd) (Fig. 3C) (14,17).

Diagonal gel analyses also show that remaining μ on NFS-5.3 exist in polymers, probably pentamers (Fig. 7A and B). They migrate as a single species which barely enters the 9% resolving gel in the nonreduced (first) dimension and is at ~70 kd in the reduced (second) dimension. In contrast, surface μ from untreated NFS-5.3 are separated into two species. One species migrates at ~190 kd in the nonreduced dimension and is at ~75 kd (μm) in the reduced dimension. The other species barely enters the 9% resolving gel in the nonreduced dimension and is at ~70 kd (μs) in the reduced dimension. Moreover, the two different-sized μ do not form mixed molecules, consistent with the observation that the membrane-form and secreted-form γ immunoglobulin heavy chains do not form mixed molecules (37,38). In summary, the above evidence strongly suggests that the remaining surface μ on NFS-5.3 are secreted-form μs.

μ heavy chains associate with other proteins to form complexes

The μ heavy chains are disulfide-linked to ω (λ_5) chains on the cell surface, but ω (λ_5) chains are poorly labeled with ^{125}I by lactoperoxidase (Fig. 7A and B) (14,17). By [^{35}S]methionine biosynthetic labeling, almost all μ chains precipitated from NFS-5.3 and 70Z/3 are disulfide-linked to ω chains (Fig. 7C

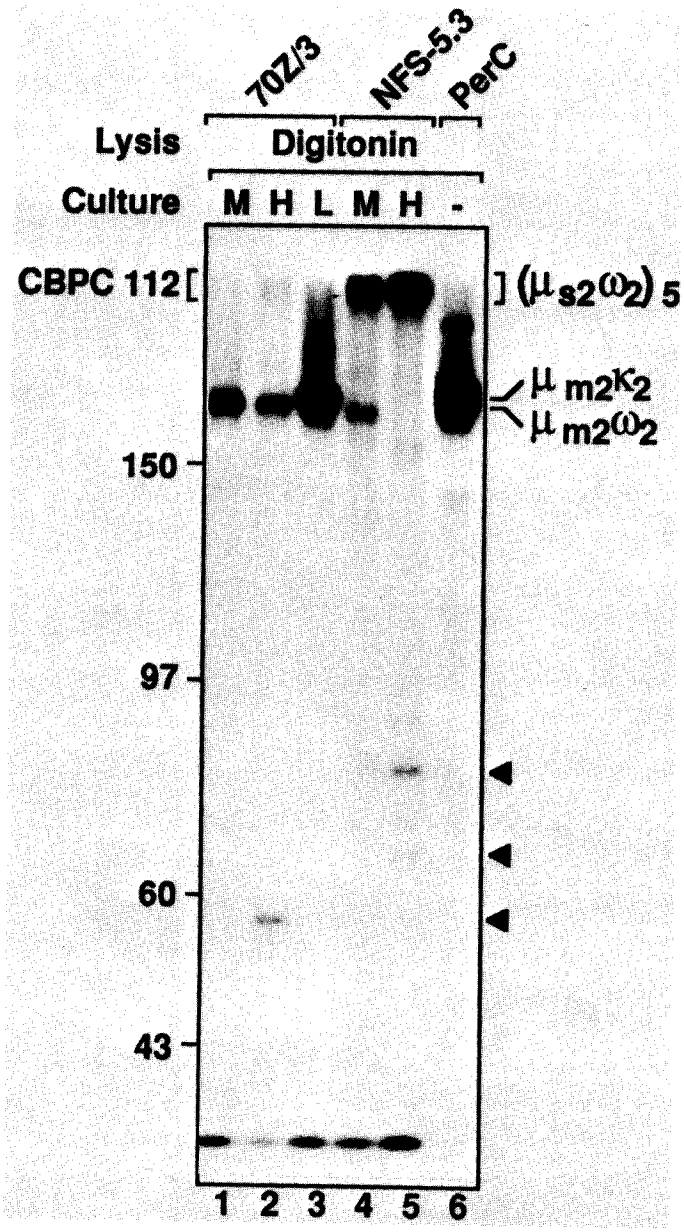

Fig. 6. The remaining μ on NFS-5.3 exist in pentamers. Anti-μ precipitates as in Fig. 3 were analyzed by 2 – 15% SDS – PAGE under nonreducing conditions. CBPC 112 is a purified pentameric IgM. The gel was stained with Coomassie blue to determine the position of pentameric IgM before autoradiography. Arrowheads point to associated proteins.

and D). In addition, surface μm from untreated 70Z/3 and NFS-5.3 are slightly smaller than monomeric IgM on nonreduced SDS – PAGE (Fig. 6). Thus, μm are disulfide-linked to ω chains to form four chain molecules of $\mu m_2\omega_2$ (11). Likewise, μs on NFS-5.3 are disulfide-linked to ω chains to form pentameric $(\mu s_2\omega_2)_5$.

Besides covalent linkage to ω (λ_5) surrogate light chains, μ heavy chains are noncovalently associated with other proteins which are detected in the anti-μ precipitates from cells lysed with digitonin (Fig. 3A and C). These additional proteins have relative molecular masses of 18, 34, and 39 kd. The 18 kd species is likely the previously identified ι ($V_{pre\text{-}B}$) (14 – 17). The 34 and 39 kd species are similar in size respectively to the IgM-α and Ig-β proteins associated with IgM on mature B cells and B cell tumors (18 – 22). Like the IgM-α and Ig-β on B cells, the 34 and 39 kd proteins form disulfide-linked heterodimers (Fig. 7). After deglycosylation, the 34 kd protein is reduced to ~24 kd, the same size as deglycosylated IgM-α (B34 or M3) (Fig. 4, lanes 8 and 10). Similarly, the deglycosylated 39 kd protein is reduced to ~30 kd, which matched the size of one of the deglycosylated IgM-associated proteins from mature B cells (Fig. 4, lane 6) (18). In addition, both IgM-α (MB-1) and Ig-β (B29) mRNAs are transcribed in pre-B cells (16,39,40) and IgM-α (MB-1) has been detected on the surface of pre-B cell line 70Z/3 (39). Therefore, the 34/39 kd heterodimers found in the μ-associated complexes on pre-B cell lines are likely identical to the IgM-α/Ig-β heterodimers associated with IgM on mature B cells.

Culturing both 70Z/3 and NFS-5.3 in the presence of heparin induces the expression of a novel 27 kd protein, termed η, which co-precipitates with surface μ in digitonin lysates (Fig. 3A) but not in NP-40 lysates (data not shown). The η chains are present as homodimers (Fig. 7B) and are not N-glycosylated (Fig. 4, lane 4). Since culturing murine spleen B cells with heparin does not induce any 27 kd protein (data not shown), this heparin-induced homodimer may be specific for the μ-associated complexes on pre-B cells.

The pentameric $(\mu s_2\omega_2)_5$ are probably anchored on the cell surface by the associated proteins. Both IgM-α and Ig-β are transmembrane proteins (16,39,40). These proteins co-precipitate with surface μ and increase the amounts simultaneously with secreted-form μs (Fig. 3A). Alternatively, the pentamers are absorbed on the cell surface after secretion due to an autoreactive specificity or by a Fc receptor-like apparatus. Pentameric IgM has been shown to be transiently present on the cell surface while on the way to be secreted (41). However, no μ heavy chains are detected in the supernatants of untreated or heparin-treated NFS-5.3 cultures after 4 h steady-state labeling (data not shown) while a lot of μ chains are synthesized during this period of time intracellularly (Fig. 3C).

Heparin stimulates IgM secretion by peritoneal Ly-1 (CD5+) B cells

In animals, heparin is synthesized and released by a variety of cells, particularly connective tissue mast cells. These mast cells that release heparin and Ly-1 (CD5+) B cells that secrete IgM tend to inhabit the same sites *in vivo*, e.g. peritoneal cavity (33, 42 – 44). The effect of heparin on pentameric IgM secretion (secreted-form μs expression) by murine peritoneal Ly-1 B cells was determined by bromelain plaquing assay since all bromelain PFCs are Ly-1 B cells (43). Peritoneal cells from BALB/c and CBA/Ca mice injected intraperitoneally with heparin show a 12- to 14-fold increase in the number of PFC over cells from control littermates that were injected with buffer solution (Table 1). Peritoneal cells cultured with heparin also have a significant increase in PFC over cells cultured in medium alone.

Similarly, endogenous heparin released from peritoneal mast cells stimulates IgM secretion by peritoneal Ly-1 B cells. Heparin in the granules of mast cells is released when mast cells are sensitized by anti-IgE antibody (33). Peritoneal cells from anti-IgE treated BALB/c mice show approximately a 7-fold

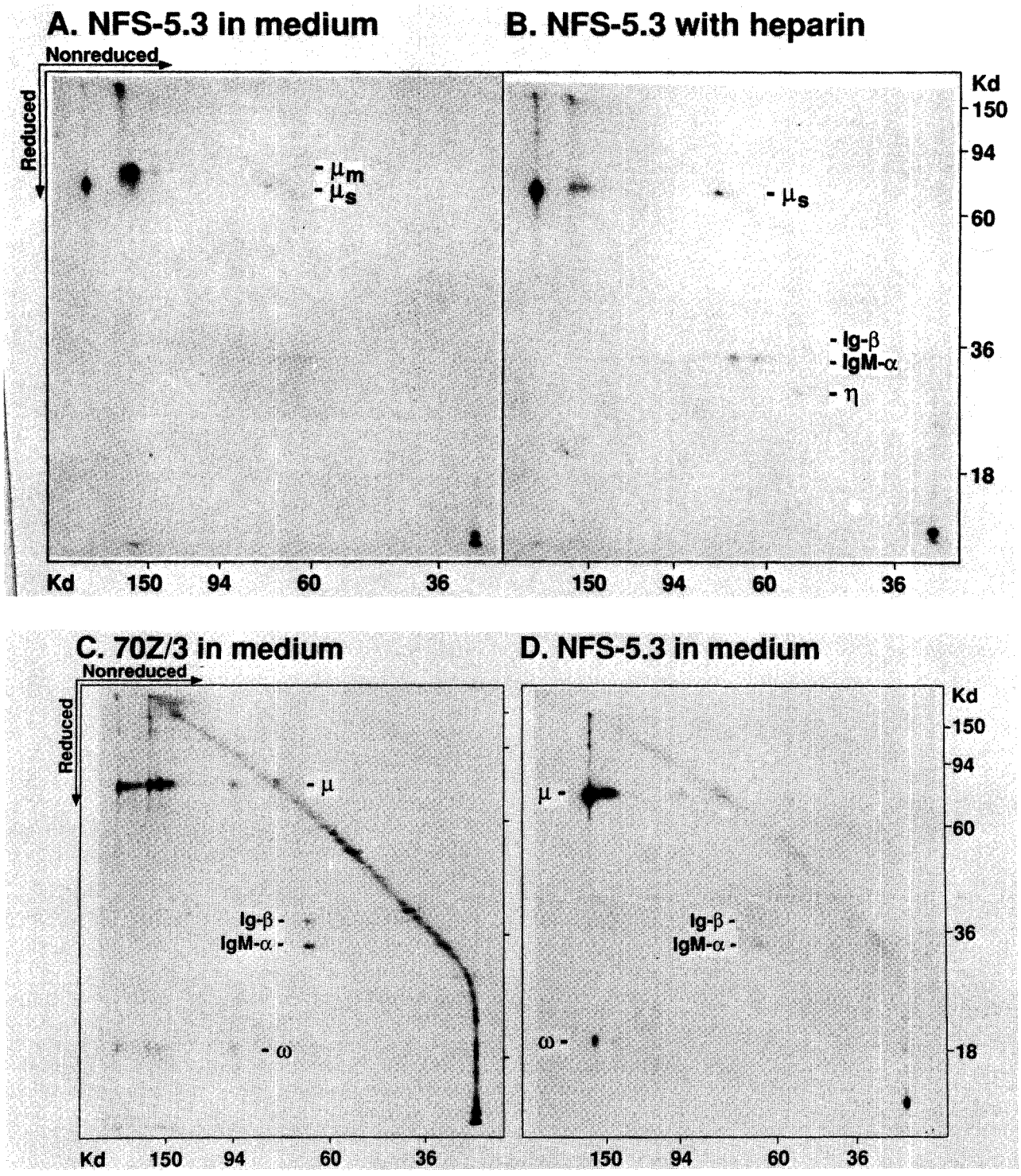

Fig. 7. Diagonal gel analyses of anti-μ precipitates from NFS-5.3 and 70Z/3 cells. Anti-μ precipitates as in Fig. 3 were analyzed in the first dimension by 9% nonreduced SDS – polyacrylamide gel and in the second dimension by 12% reduced SDS – PAGE. (A) and (B) ^{125}I-surface-labeled precipitates; (C) and (D) [^{35}S]methionine biosynthetic labeled precipitates.

increase in PFC over peritoneal cells from littermates treated with isotype-matched control antibody (Table 1). Peritoneal cells cultured with anti-IgE antibody also show a 3- to 4-fold increase in PFC over cells cultured with control antibody.

Anti-IgE-sensitized mast cells also release Th2 cell-type lymphokines like IL-3, IL-4, IL-5, and IL-6 (45). Among these lymphokines, IL-5 has been shown to stimulate IgM secretion by Ly-1 B cells (26,27). This may contribute to the IgM secretion

Table 1. Heparin stimulates IgM secretion by peritoneal Ly-1 B cells

Strain	Treatment[a]		Bromelain PFC per million PerC
BALB/c	medium	*in vivo*	20 ± 5
	heparin	*in vivo*	278 ± 13
	control Ab	*in vivo*	110 ± 9
	anti-IgE Ab	*in vivo*	694 ± 20
CBA/Ca	medium	*in vivo*	10 ± 3
	heparin	*in vivo*	119 ± 14
CBA/Ca	medium	*in vitro*	22 ± 6
	heparin	*in vitro*	58 ± 8
	control Ab	*in vitro*	32 ± 7
	anti-IgE Ab	*in vitro*	108 ± 15

[a]For *in vivo* treatment, mice of the same litter were intraperitoneally injected with the following 24 h prior to bromelain plaquing assay: medium, deficient RPMI, heparin, 300 μg/mouse (~30 g); anti-IgE Ab, 30 μg/mouse; or isotype-matched control Ab, 30 μg/mouse. At least two mice were used for each treatment. For *in vitro* treatment, peritoneal washout cells (PerC) from mice of the same litter were pooled and cultured as follows for 24 h prior to bromelain plaquing assay: medium, normal RPMI; heparin, 100 μg/ml; anti-IgE Ab, 5 μg/ml; or isotype-matched control Ab, 5 μg/ml. Bromelain PFC is normalized to per million peritoneal cells since both heparin and anti-IgE treatment do not alter the percentage of Ly-1 B cells in peritoneal cavity or in culture. Each PFC number is the average of two independent experiments in which four duplicates were assayed for each sample.

by more Ly-1 B cells with anti-IgE treatment than with commercial heparin treatment. To determine the relative contribution of heparin and IL-5 in stimulating IgM secretion by normal peritoneal Ly-1 B cells, protamine, a heparin antagonist, was used to block the effect of heparin on IgM secretion in culture. However, very few cells survived the culture. Thus, the relative contribution of IgM secretion stimulated by heparin released from anti-IgE sensitized mast cells remains to be determined.

Discussion

Studies presented here clearly show that heparin has novel activities that can alter the expression of different forms of immunoglobulin μ heavy chains and their associated proteins by pre-B cell lines and normal Ly-1 B cells. These findings are consistent with evidence demonstrating that heparin influences cell differentiation or function of many cell types (46–48). Our studies suggest new roles specific for heparin not other proteoglycans such as chondroitin sulfate and heparin sulfate. Although these proteoglycans all consist of glycosaminoglycans attached to peptide cores, they differ in the type of hexosamine, location of sulfation, degree of sulfation, and extent of epimerization of the uroic acid (33). Particularly, they are synthesized and released by different cell types that are localized in different tissues *in vivo*. This tissue-specific heparin distribution coincides with its potential physiological functions.

For example, connective tissue mast cells that release heparin and Ly-1 B cells that can be rapidly stimulated by heparin to secrete IgM tend to inhabit the same sites *in vivo*, e.g. peritoneal cavity, while mucosal mast cells that release chondroitin sulfate reside in gastrointestinal mucosa (33,43,44). Thus, heparin could contribute to the stimulation of IgM production by Ly-1 B cells, which account for roughly half the serum IgM in normal mice (42). Furthermore, if Ly-1 B cells (like mast cells) migrate to the site of an inflammation, heparin could stimulate a local production of IgM by these B cells, many of which produce polyreactive antibodies that could be particularly useful for removing allergens or other inflammation-inducing substances.

Our studies also demonstrate that μ heavy chains on pre-B cells noncovalently associate with additional proteins besides the previously identified ι ($V_{pre\text{-}B}$). Two of these proteins are probably the IgM-α/Ig-β heterodimers which associate with IgM on mature B cells (18–21). The presence of the IgM-α/Ig-β heterodimers in the μ-associated complexes further suggests that these complexes play a role in regulating pre-B cell differentiation. The μ-ω immunoglobulin-like molecules are potentially capable of recognizing ligands and thus could function as receptors. The IgM-α/Ig-β heterodimers are likely involved in transducing μ-ω-initiated signals in ligand-stimulated pre-B cells as they are probably involved in transducing IgM-initiated signals in antigen-stimulated B cells (18,21,39,40,51,52). Thus, pre-B cells have a mechanism, similar to that of mature B cells, which can sense the presence of relevant ligands and signal events in pre-B cell differentiation.

Thus, heparin may also be influential in pre-B cell differentiation. If heparin reduces the expression of the membrane-form μm on pre-B cells to the same extent that it reduces membrane-form μm expression on pre-B cell lines, it could markedly diminish the capacity of ligand recognition by pre-B cells and thus impair ligand-triggered differentiation. On the other hand, if it increases the expression of pentamers of secreted-form μs on pre-B cells as it does on NFS-5.3, it could provide means for ligand to signal a different differentiation event. Since pentamers bind ligands with a higher avidity, their presence should be quite advantageous, particularly in pre-B cells which express only a small amount of μ on the surface.

The production of secreted-form μ chains by pre-B cells has been documented previously in studies showing: (i) that pre-B cell lines and immature B cell lines produce mRNA for secreted-form μ (2–4); (ii) that the secreted-form μ heavy chain is synthesized in the cytoplasm of pre-B cell lines (5–8); (iii) the μ heavy chains precipitated from pre-B cell lines are linked by disulfide bonds to ω surrogate light chains (11,14,15). Studies presented here demonstrate that a small amount of secreted-form μ reaches NFS-5.3 cell surface and is present in pentameric form.

Immunoprecipitation analyses show that roughly equal amounts of the secreted-form μ chains and the membrane-form μ chains are present in NFS-5.3 cells under normal culture conditions. Culturing with heparin removes the membrane-form μ completely and increases the amount of the secreted-form μ. This secreted-form μ is clearly on the cell surface since it is detectable by immunoprecipitation after surface iodination and the associated proteins co-precipitate and increase with this μ chain. Furthermore, it is detectable on viable cells by immunofluorescence staining and FACS analysis. Thus, studies with heparin-cultured cells definitely demonstrate that a portion of the secreted-form μ produced by pre-B cell line NFS-5.3 is present on the cell surface.

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Abbreviations

APC	allophycocyanin
FACS	fluorescence-activated cell sorter
γ-IFN	γ-interferon
IL-1, -4, -5	interleukin 1, 4, 5
LPS	lipopolysaccharide
μ	immunoglobulin μ heavy chain
μm	membrane-form immunoglobulin μ heavy chain
μs	secreted-form immunoglobulin μ heavy chain
PerC	peritoneal washout cells
PFC	plaque-forming cells

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