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# BIOSYNTHESIS AND TWO-DIMENSIONAL GEL ANALYSIS OF IgM AND IgD RECEPTORS

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## INTRODUCTION

The biosynthesis and secretion of the serum immunoglobulins are now reasonably well understood.[1] In contrast, there is a paucity of information on the biosynthesis of lymphocyte surface immunoglobulins. A mature plasma cell may devote 40% of its total protein synthesis to immunoglobulin, and newly synthesized immunoglobulin may attain constant specific activity within 2 hours of addition of radioactive amino acids.[1] On the other hand, IgM and IgD receptors turn over relatively slowly,[2] and represent a minor fraction of the total cell proteins.

In this paper, we describe the biosynthesis of IgM and IgD receptors, using non-equilibrium pH gradient gel electrophoresis (NEPHGE) followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), a technique that is capable of extremely high resolution analysis of complex protein mixtures.[3-5] We show that IgM exists in at least three discrete forms: an intracellular precursor, a secreted form, and a surface form. We also provide evidence for a cytoplasmic precursor form of IgD.

## MATERIALS AND METHODS

Biosynthetic labeling with $^{35}$S-methionine,[5] surface radio-iodination,[5] immunoprecipitation,[5] and two-dimensional gel analysis[4] were as described, except that the pH of the NP-40 lysis buffer was 8.0 to allow binding of $IgG_1$ to staphylococcal protein A.[6] Wash buffers were 0.1M tris pH 8 containing 0.4M NaCl, resulting in less nonspecific binding. Antibodies used were polyvalent rabbit anti-mouse immunoglobulin and monoclonal anti-IgD allotype antibodies.[7]

## RESULTS

Spleen cells from 5-day old CSW mice were cultured for 1 hr with 200 μCi $^{35}$S-methionine, washed, and cultured for a further 7 hr in the presence of cold methionine. At 1 hr and 8 hr, aliquots of cells were lysed with NP-40, and samples of the culture supernates were taken. Radioactive immunoglobulin was precipitated with a polyvalent serum, and analyzed on 2-D gels. Results are shown in Fig. 1.

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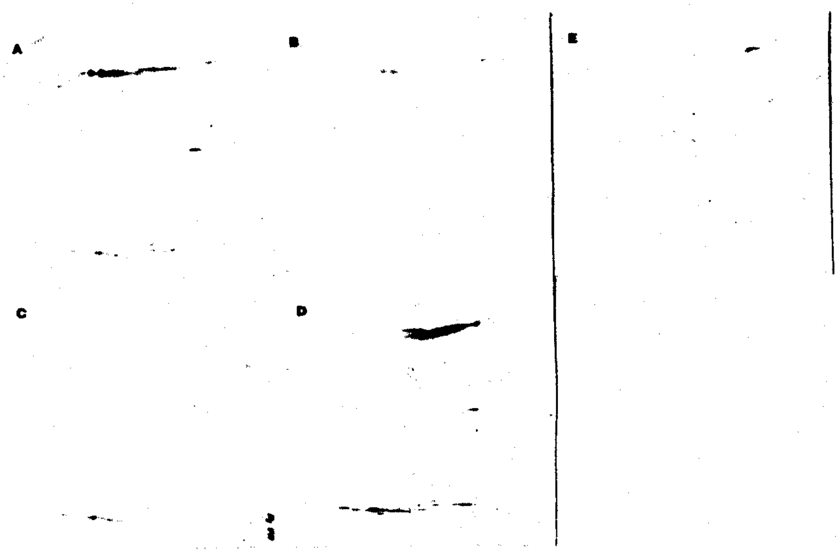

Fig. 1. Two-dimensional gel analysis of IgM from 5-day old mice. A, 1 hr cell lysate; B, 8 hr lysate; C, 1 hr medium; D 8 hr medium; E, surface iodination. The acidic end of the gel is to the right, and SDS electrophoresis is from top to bottom. Exposure time was 21 days.

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Fig. 1-A shows that IgM molecules can be resolved into three distinct populations, with increasing acidity and decreasing mobility in SDS-PAGE. These will be referred to as $\mu_1$, $\mu_2$ and $\mu_3$, respectively. At 1 hr (Fig. 1-C), a trace of $\mu_2$ is seen in the supernate, while after 7 hr chase, all the $\mu_2$ is in the supernate (Fig. 1-D). Following the chase, the $\mu_1$ band is much less prominent, while the $\mu_3$ band remains unchanged.

The chase from $\mu_1$ to $\mu_2$, and the presence of $\mu_2$ (but not $\mu_1$ or $\mu_3$) in the supernate, are consistent with the notion that $\mu_1$ represents a cytoplasmic precursor for the secreted $\mu_2$ form. The shift of $\mu_1$ to a more acidic and slower $\mu_2$ chain is consistent with the addition of neutral sugars and sialic acid during passage through the secretory apparatus of the cell.

The $\mu_3$ band, which represents the most acidic and slowest moving form of IgM, probably represents the surface form, since (a) its mobility in both dimensions is identical to that of IgM receptors labeled by surface iodination (compare Figs. 1-B and 1-E), (b) $\mu_3$ chains are not secreted (Figs. 1-C and 1-D) and (c) the turnover of $\mu_3$ appears to be relatively slow (Fig. 1-B).

## DISCUSSION

IgM heavy chains are synthesized on the rough endoplasmic reticulum. They rapidly acquire "core" sugars (glucosamine and mannose), are transported to the smooth endoplasmic reticulum, and are secreted via the Golgi apparatus. Shortly before (or during) secretion they acquire galactose, fucose and sialic acid.[1] For a detailed review of the structure of mammalian cell surface oligosaccharides, the reader is referred to a paper by Kornfeld and Kornfeld.[9]

The stepwise transition of μ chains to the more acidic and more slowly moving forms observed in our experiments is consistent with the above formulation, at least for secretory IgM. However, we have not yet been able to show whether surface and secreted IgM have a common cytoplasmic precursor. This key point is currently under investigation. Surface IgM migrates more slowly than secreted IgM in SDS-PAGE,[10,11] and also differs from secreted IgM in its ability to bind detergent[12] (Parkhouse et al, this volume). Our data provide evidence for a discrete surface form of μ chain, which is more acidic than either secreted μ or its precursor. A similar progression is seen in the B cell leukemia BCL-1 (M. Knapp and P. Jones, personal communication). Our data do not allow us to say whether the differences are due to the polypeptide chain or carbohydrate (additional sialic acid) or both.

Until now, the biosynthetic labeling of IgD has not been achieved, at least partly because of its slow turnover. The demonstration of IgD biosynthesis in our experiments depended on the use of an isotope that is available at very high specific activity ($^{35}$S-methionine; 800 Ci/millimole), and a detection system capable of detecting as little as 1 cpm radioactivity.[5] The use of monoclonal antibodies to IgD[7] assured the specificity of precipitation.

Our results suggest the presence of a more rapidly migrating, more basic cytoplasmic IgD precursor molecule. However, due to the great susceptibility of IgD to proteolysis,[13] identification of this molecule as a precursor is tentative. Testing of a precursor-product relationship will require pulse-chase experiments. The slow turnover of IgD, together with the significant death of normal mouse lymphocytes in culture, will render this form of analysis difficult. The availability of tissue culture adapted mouse B cell leukemias will facilitate these studies.

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Biosynthesis of IgD

The demonstration of the biosynthesis of IgD proved more difficult, perhaps due to its slow turnover. Although some incorporation was observed with a 1 hr pulse and 7 hr chase, results were much more satisfactory with a 4 hr labeling period and 4 hr chase. Results are shown in Fig. 2.

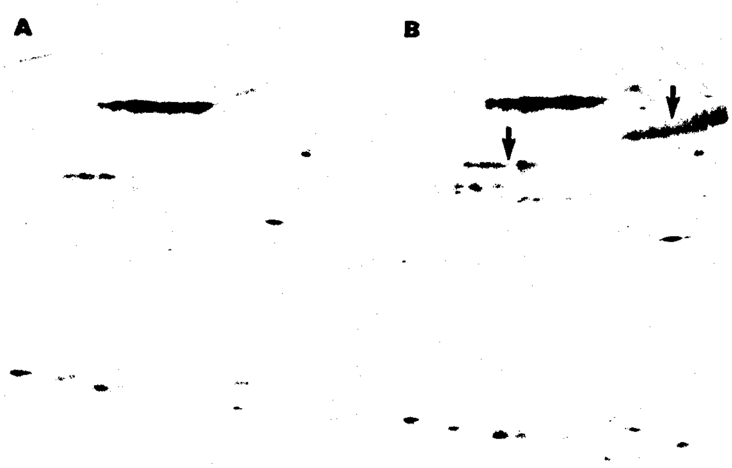


Fig. 2. Two-dimensional gel analysis of IgD biosynthesis. Spleen cells from 8 wk old BALB/c mice were labeled with $^{35}$S-methionine (text), and lysed with NP-40. Half the lysate was reacted with monoclonal anti-Ig-5b (inappropriate allotype control) and half with anti-Ig-5a (correct allotype). The bound products were analyzed in panels A and B, respectively. Delta chains are marked with arrows. Exposure time was 10 days.

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Since the lysates were not precleared with staphylococci, some IgM (a small minority of the secretory $\mu_2$ form) and IgG (the majority of that synthesized) is seen in both gels (Fig. 2). It is known that a minor fraction of mouse IgM, and most mouse IgG, bind to protein A.[8] However, in Fig. 2-B, two additional bands are seen (arrows). The more acidic and larger form has the same mobility as surface labeled IgD receptors, although we have not yet proved that this molecule is present on the cell surface. The more basic band probably represents an incompletely glycosylated cytoplasmic precursor for surface IgD, although we have not excluded the possibility that it is a breakdown product.