a band of M_r ~250,000 (250K) was identified under non-reducing conditions. A single band of M_r ~140K was generated under reducing conditions for disulphide bonds (Fig. 2). Internal labelling of 18.81 cells with ^{35}S-methionine, followed by sizing gel analysis of BP-1-reactive material, also revealed a molecule comprising disulphide-linked subunits of ~140K. The 140K band could be detected when the BP-1 antibody was used to identify reactive molecules after electrophoresis of an 18.81 cell lysate (reducing conditions) and transfer of the separated proteins to a nitrocellulose membrane[28], indicating relative stability of the antigenic determinant on individual subunits. When BP-1-reactive molecules on 18.81 cells were isolated, reduced and treated with endoglycosidase F (Endo F) to remove *N*-linked oligosaccharides[29], the M_r of the subunits was reduced to ~115K (Fig. 2*B*).

BP-1-reactive molecules of identical size and composition were also found in normal bone marrow (Fig. 2*A*) and in 18.81, 70 Z and 38C-13 cell lines (data not shown). Immunochemical analysis failed to reveal BP-1-reactive molecules on cells from spleen, brain and kidney or on cell lines representative of later stages in B-cell differentiation.

The restricted expression of the BP-1 alloantigen suggests that it could serve as a cell adhesion molecule for early B-lineage cells, or it could be a receptor for a unique induction signal. Noteworthy in the latter regard is the relatively high expression of BP-1-reactive molecules by transformed as opposed to normal pre-B cells. Whatever the functional role of this surface molecule, the BP-1 antibody provides an incisive probe with which to explore early events in the differentiation of normal and neoplastic B-cell clones. The alloimmunization strategy used to produce this antibody could be useful for identifying a variety of highly conserved molecules involved in cellular differentiation.

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Chloroquine and ammonium chloride prevent terminal glycosylation of immunoglobulins in plasma cells without affecting secretion

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The generation of an acidic *p*H in intracellular organelles is required for several membrane and protein recycling processes. For instance, the internalization of ligands by receptor-mediated endocytosis is followed by the development of an acidic *p*H inside endosomes; this allows dissociation of the ligand, which is then transported to the lysosomes, from the receptor, which is recycled to the cell surface[1–4]. There is evidence that part of this recycling process involves the distal region of the Golgi complex, where terminal glycosylation occurs: when the plasma membrane transferrin receptor is desialylated by neuraminidase treatment, it acquires new sialic acid molecules after endocytosis and before cell-surface re-expression[5]. Golgi membranes have been shown to contain a proton pump[6] and the distal Golgi cisternae appear to have an acidic content[7]. Here, we have studied the effects of chloroquine and ammonium chloride, which raise the *p*H of acidic intracellular compartments[8], on the processing and secretion of immunoglobulins by plasma cells. Sialic acid transfer to terminal galactose residues, a reaction known to occur in the distal Golgi shortly before secretion[9], is completely and rapidly inhibited in the presence of these drugs, without significant modification of the secretion rate. This effect is accompanied by a dilatation of the Golgi cisternae and is not rapidly reversible.

Biosynthetically labelled immunoglobulin μ-chains are secreted in the form of pentameric IgM by mouse plasma cells; when fully reduced and alkylated, they have a retarded mobility on SDS-polyacrylamide gel electrophoresis (PAGE) compared

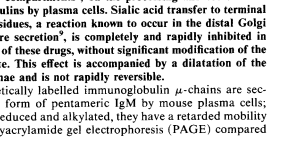

Fig. 1 *a*, Plasma cells, elicited in spleen cell cultures by *Escherichia coli* lipopolysaccharide[18], were labelled biosynthetically with 100 μCi ml^{-1} ^{35}S-methionine for 1 h, the lysates and media were prepared and immunoglobulins were immunoprecipitated and analysed by SDS-PAGE[19] (17.5% polyacrylamide). Chloroquine (CQ) was at 100 μM and ammonium chloride (NH_4Cl) at 10 mM. μ, μ-chains; L, light chains. *b*, Plasma cells were labelled biosynthetically for 1 h with 100 μCi ml^{-1} ^{3}H-galactose in Dulbecco's modified Eagle's medium containing 0.1 mg ml^{-1} glucose and 10% dialysed fetal calf serum, in the presence or absence of 100 μM chloroquine. Secreted μ-chains were on the same gel, analysed as in *a*. μ_{ext}, Mobility of secreted normal μ-chains; μ_{int}, mobility of intracellular incompletely glycosylated μ-chain precursors.

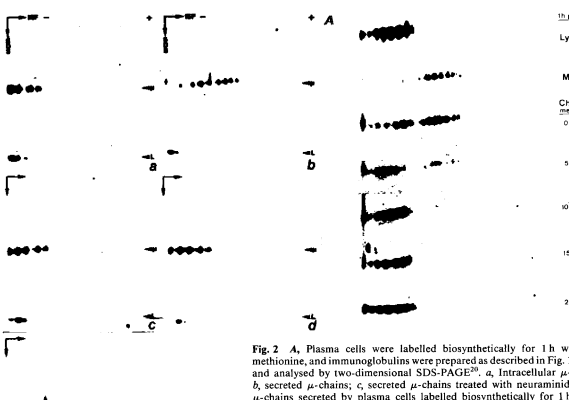

Fig. 2 *A*, Plasma cells were labelled biosynthetically for 1 h with ^{35}S-methionine, and immunoglobulins were prepared as described in Fig. 1 legend and analysed by two-dimensional SDS-PAGE[20]. *a*, Intracellular μ-chains; *b*, secreted μ-chains; *c*, secreted μ-chains treated with neuraminidase[9]; *d*, μ-chains secreted by plasma cells labelled biosynthetically for 1 h in the presence of 100 μM chloroquine; *e*, μ-chains secreted by plasma cells incubated for 1 h in the presence of 100 μM chloroquine, then for 4 h in the absence of chloroquine and finally labelled for 1 h in the absence of chloroquine. *B*, Plasma cells were labelled biosynthetically for 1 h, then chased in the presence of 100 μM chloroquine. The medium was collected and replaced at 5-min intervals and the immunoglobulin chains immunoprecipitated and analysed as described in Fig. 1 legend. Only the μ-chains are shown.

with intracellular μ-chains, due to the complete glycosylation of their oligosaccharide side chains (ref. 9 and Fig. 1). When plasma cells were labelled in the presence of chloroquine or ammonium chloride, the secreted μ-chains had the same mobility as their intracellular precursors (Fig. 1*a*), indicating that the glycosylation process was impeded. To determine the nature of the missing sugar residues, plasma cells were labelled biosynthetically in the presence of ^{3}H-galactose, the penultimate sugar residue in complex-type *N*-linked oligosaccharides. Figure 1*b* shows that the presence of chloroquine did not prevent the addition of ^{3}H-galactose to μ-chains and that the galactose-labelled chains secreted by the chloroquine-treated cells also had a higher mobility. The degree of sialylation of μ-chains was then assessed by two-dimensional SDS-PAGE (Fig. 2*A*). Heavy μ-chains secreted by plasma cells have more acidic *p*Is (Fig. 2*Ab*) than do intracellular μ-chains (Fig. 2*Aa*) (the vast majority of which have not undergone terminal glycosylation, topologically and chronologically a very late event in the intracellular pathway[9]). This difference in *p*I was totally suppressed after treatment of secreted immunoglobulins with neuraminidase (Fig. 2*Ac*). Secreted μ-chains synthesized in the presence of chloroquine (Fig. 2*Ad*) or ammonium chloride (not shown) had the same mobility as unsialylated μ-chains.

Pulse-chase experiments showed that the abolition of immunoglobulin sialylation by these drugs is induced very rapidly, but is not rapidly reversible and has no effect on the kinetics of immunoglobulin secretion. Plasma cells were first labelled biosynthetically for 60 min to allow homogeneous labelling of the immunoglobulins present along the secretory pathway, then placed in a chase medium containing chloroquine; the medium was collected and replaced at 5-min intervals. Two-dimensional SDS-PAGE showed that μ-chains lacking sialic acid could be detected within 5 min of chloroquine addition, and that sialylated μ-chains were dramatically reduced after 10 min of chloroquine treatment (Fig. 2*B*). To explore the reversibility of this inhibition, plasma cells were treated for 1 h with chloroquine without labelling, then placed in a medium without chloroquine for 4 h and finally pulse-labelled for 1 h: the secreted μ-chains were still not sialylated (Fig. 2*Ae*), indicating that the effect of the drug is not rapidly reversible. Similar observations were made with ammonium chloride (not shown). That this inhibition of sialylation has no significant effect on the kinetics of immunoglobulin secretion was found by labelling plasma cells for 30 min then chasing for periods of various length, in the absence or presence of chloroquine; analysis of the μ-chains remaining in the cells or secreted in the medium at these various intervals (Fig. 3) showed that the presence of the drug did not significantly modify the secretion rate.

To explore the possibility that the inhibition of sialylation resulted from a greater sensitivity of sialyltransferase than galactosyltransferase to changes in *p*H or to the presence of high concentrations of chloroquine, plasma cell membranes were

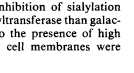

Fig. 3 Plasma cells were labelled biosynthetically with ^{35}S-methionine for 30 min (P), then chased (Ch) for 40 or 90 min, in the presence or absence of 100 μM chloroquine (CQ). The μ-chains present in the lysates of, and secreted by 2×10^6 cells were immunoprecipitated and analysed by SDS-PAGE.

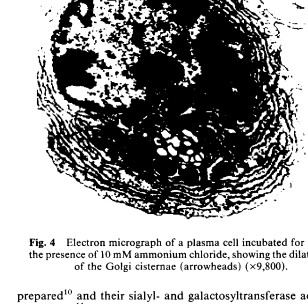

Fig. 4 Electron micrograph of a plasma cell incubated for 1 h in the presence of 10 mM ammonium chloride, showing the dilatation of the Golgi cisternae (arrowheads) (×9,800).

prepared[10] and their sialyl- and galactosyltransferase activities determined[11] at *p*Hs between 5.8 and 7.0, in the presence or absence of 10 mM chloroquine. The activity of neither enzyme was modified within this *p*H range; chloroquine at this high concentration decreased the sialyltransferase activity by 30–40% and the galactosyltransferase activity by 60%. Thus, defective sialylation does not result from a simple differential effect of *p*H or chloroquine on the terminal sugar transferase activities.

Plasma cells incubated for 1 h in the presence of chloroquine or ammonium chloride showed two types of ultrastructural alterations: a dilatation of some of the Golgi cisternae (Fig. 4), which were usually positive for thiamine pyrophosphatase activity (not shown), and the presence of very large, clear vacuoles with a scattered distribution, which were observed only in the presence of 100 μM chloroquine, and which have been described in similar conditions in other cells, such as fibroblasts[12]. Golgi alterations were still present 3 h after removal of the drug, while the large vacuoles observed in 100 μM chloroquine, which are probably swollen lysosomes or endosomes, had disappeared. Thus, only the Golgi dilatation is consistently correlated with the metabolic abnormality.

It is not clear why drugs that raise the *p*H of acidic intracellular compartments, such as ammonium chloride, chloroquine and monensin, induce an irreversible, probably *trans*, dilatation of the Golgi complex. In the case of monensin, a carboxylic membrane ionophore, the perturbations of the Golgi complex are probably both structurally and functionally more complex since, in addition to an extensive vacuolation of the Golgi cisternae, secretion of immunoglobulins by plasma cells is blocked[13]. In the case of ammonium chloride and chloroquine, it is not obvious why the more limited Golgi alteration is accompanied by inhibition of sialic acid addition but not of galactose addition or immunoglobulin secretion, even though it is usually considered that the two transferases share the same cisternal localization[11,14–16]. However, in highly secretory cells such as plasma cells, there is an enormous and very rapid vesicular traffic between the distal Golgi cisternae and the cell surface. Sialyltransferase, being the last enzyme to act in the processing of immunoglobulin carbohydrate side chains, may lie still more distally along the secretory pathway; indeed, it has recently been shown in hepatocytes that sialyltransferase is present not only in the Golgi *trans* cisternae, but also more distally in a complex network continuous with these cisternae[17] and in which no galatosyltransferase has been detected[15]. If a comparable structure exists in plasma cells, it is conceivable that the enzyme should be constantly carried back towards the distal Golgi cisternae, as part of a membrane protein recycling process. This may require the constant generation of a *p*H gradient to allow the persistence of a steady enzyme gradient. Disruption of the *p*H gradient may allow sialyltransferase and perhaps other membrane proteins (for instance, the Golgi proton pump itself) to remain at the cell surface irreversibly, explaining the persistence of the Golgi alterations and of the lack of sialylation seen in the present experiments. Since the *trans* part of the Golgi complex is involved in the intracellular traffic of endocytosed plasma membrane receptors[5], its involvement in the recycling of other membrane-associated proteins may explain the effects of intracellular *p*H-disrupting drugs in highly secretory cells.

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Structure of DNase I at 2.0 Å resolution suggests a mechanism for binding to and cutting DNA

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Bovine pancreatic deoxyribonuclease I (DNase I), an endonuclease that degrades double-stranded DNA in a nonspecific but sequence-dependent manner[1–4], has been used as a biochemical tool in various reactions, in particular as a probe for the structure of chromatin and for the helical periodicity of DNA on the nucleosome and in solution[5–10]. Limited digestion by DNase I, termed DNase I 'footprinting', is routinely used to detect protected regions in DNA–protein complexes[11]. Recently, we have solved the three-dimensional structure of this glycoprotein (relative molecular mass 30,400) by X-ray structure analysis at 2.5 Å resolution[12] and have subsequently refined it crystallographically at 2.0 Å (ref. 26). Based on the refined structure and the binding of Ca^{2+}–thymidine 3′,5′-diphosphate (Ca-pTp) at the active site[12], we propose a mechanism of action and present a model for the interaction of DNase I with double-stranded DNA that involves the binding of an exposed loop region in the minor groove of B-DNA and electrostatic interactions of phosphates from both strands with arginine and lysine residues on either side of this loop. We explain DNase I cleavage patterns in terms of this model and discuss the consequences of the extended DNase I–DNA contact region for the interpretation of DNase I footprinting results.

In our earlier paper[12] we described the binding of Ca-pTp to DNase I the binding of Ca-pTp to DNase I. The di-*p*-nitrophenylester of pTp is a substrate for DNase I (ref. 13). Similar