Brief Definitive Report

CHLOROQUINE AFFECTS BIOSYNTHESIS OF Ia MOLECULES BY INHIBITING DISSOCIATION OF INVARIANT (γ) CHAINS FROM α-β DIMERS IN B CELLS

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Ia molecules are integral cell surface membrane glycoproteins that are expressed primarily by antigen-presenting cells, and that restrict immune recognition of foreign antigens by helper T lymphocytes (reviewed in 1). In the cytoplasm, the Ia molecular complex is formed by the noncovalent association of the highly polymorphic α and β chains, encoded by class II genes of the major histocompatibility complex (MHC), and the nonpolymorphic γ (or invariant) chain (2, 3), encoded by gene(s) unlinked to the MHC (reviewed in 4). Association occurs immediately after translation (5); γ chains then dissociate concurrently to terminal carbohydrate processing (6), so that on the cell surface, Ia molecules consist of α-β dimers (2–4). The fate of γ chains after dissociation, as well as their overall role in the biology of Ia molecules, is uncertain (4, 5). An understanding of the details of Ia biosynthesis appears necessary for a deeper insight into the molecular mechanisms of immune recognition. In analogy with studies of other intracellular protein transport systems (7, 8), we have used the lysosomotropic drug chloroquine to investigate some aspects of Ia biosynthesis.

Materials and Methods

Cell Lines and Antisera. The Burkitt's lymphoma lymphoblastoid cell line (LCL), Raji, and the Epstein-Barr virus–transformed B LCL, LG-2 and 721, were obtained from A. Theofilopoulos (Research Institute of Scripps Clinic), R. Gatti (University of California, Los Angeles), and F. Bach (University of Minnesota, Minneapolis, MN), respectively. Monoclonal antibodies (mAb) used were VIC-Y1, specific for the human Ia γ chain (3), and Q5/13, reactive against the β chains of most human Ia molecules (HLA-DR and DQ) (3).

Cell Labeling, Immunoprecipitation, and Electrophoresis. Cells were incubated with or without drugs (10^{-6} M chloroquine; 10^{-2} M NH_4Cl) at 37°C in methionine-free RPMI 1640 medium (Gibco Laboratories, Grand Island, NY), supplemented with 3% dialyzed fetal calf serum. At the indicated concentrations of drugs, cells were >90% viable after 48 h, as determined by trypan blue exclusion. After 60 min, [^{35}S]methionine (sp act 600 Ci/M; Amersham Corp., Arlington Heights, IL) was added at 1.0 mCi/ml. Cells were pulse-labeled for indicated times at 37°C, washed once, and resuspended in regular RPMI 1640 medium containing the appropriate drug and an excess of methionine (5 mM).

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Aliquots were harvested at various chase times, quickly chilled, washed three times in ice-cold 10 mM Tris-HCl, 150 mM NaCl, pH 7.5, and solubilized. In pilot experiments, trichloroacetic acid precipitation and scintillation counting was performed on samples from each chase time point to insure that no further incorporation of radiolabeled methionine occurred after the initial pulse. Cell lysates were prepared as described (3), in 10 mM Tris-HCl, 150 mM NaCl, 2 mM phenylmethylsulphonylfluoride, pH 8.5, containing 2% of the nonionic detergent Renex 30 (Ruger Laboratories, Piscataway, NJ). Immunoprecipitations were carried out as described (3), by overnight incubation of cell lysates with immunoabsorbents prepared by absorption of mAb to protein A–Sepharose beads (Sigma Chemical Co., St. Louis, MO). After elution in 8 M urea at room temperature, samples were analyzed by two-dimensional electrophoresis, consisting of nonequilibrium pH electrophoresis on tube gels in the first dimension, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on 10% acrylamide slab gels (9). Gels were impregnated with 2,5-diphenyloxazole, dried, and exposed for indicated times to Kodak XAR-5 X-ray film at −70°C (9).

Results and Discussion

The effects of lysosomotropic agents on biosynthesis of Ia molecules were examined in the Ia^+ human B LCL, Raji, LG-2, and 721, with comparable results. Ia antigens were analyzed by immunoprecipitation with mAb, followed by two-dimensional nonequilibrium pH gradient electrophoresis (NEPHGE). When cells were pulse-labeled for 1 h with [^{35}S]methionine and then chased overnight (16 h), Ia molecules reactive with an anti-β chain mAb (Q5/13) were found to be composed mainly of fully processed α and β chains, with very little γ chain associated (Fig. 1). In contrast, immunoprecipitates (IP) of the same cells with an anti-γ chain mAb (VIC-Y1) showed mainly γ chains, a small amount of sialylated γ chains (γ_s), and very faint spots corresponding to immature α and β chains (Fig. 1). The divergence of anti-β and anti-γ NEPHGE patterns indicated that, under the conditions of pulse-chase used, most Ia oligomers had chased into mature forms dissociated from γ chains. However, in cells labeled under identical conditions in the presence of chloroquine, the NEPHGE patterns of Q5/13 and VIC-Y1 IP were strikingly altered (Fig. 1). The spots corresponding to γ_s chains had become extremely intense, both in Q5/13 and VIC-Y1 IP. The level of γ chains (based on intensity of spots) was also higher in Q5/13 IP, and almost identical to the amounts found in the VIC-Y1 IP. Notable also was the presence of mature α and β chains in VIC-Y1 IP. These results suggested that, in the presence of chloroquine, the processing of α-β-γ oligomers into α-β dimers and dissociated γ chains was much less efficient, so that both anti-β and anti-γ reacted with oligomers of similar subunit composition, as opposed to the diverging patterns seen in control cells (Fig. 1). Treatment of cells with NH_4Cl resulted in similar, yet less pronounced effects (Fig. 1).

To determine whether chloroquine simply induced sluggishness in the Ia biosynthetic pathway or was selective for a specific processing step, we analyzed cells at various times in a pulse-chasing experiment by immunoprecipitation with Q5/13 (anti-β). The most representative time points are shown in Fig. 2. At 15 min, Ia oligomers from control cells consisted mainly of γ chains and immature α and β chains. At 30 min, terminally glycosylated α and β chains, as well as γ_s, began to appear, while the γ chain spot decreased in intensity (Fig. 2, *CTRL*). At 240 min, both γ and γ_s spots were fainter, while immature α and β chains kept chasing into mature forms. Note that γ chains never entirely chase into their γ_s

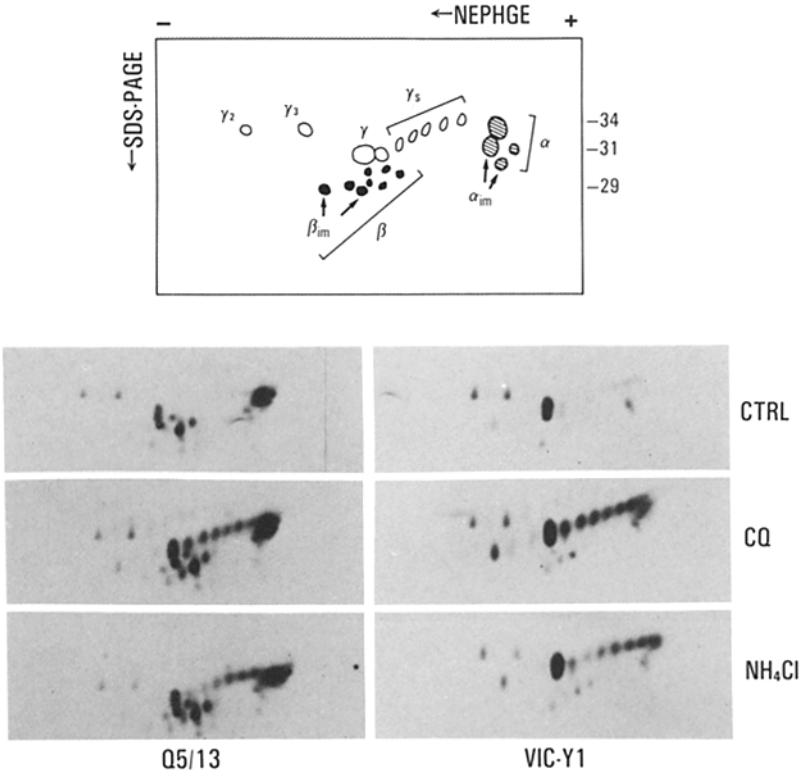

FIGURE 1. Two-dimensional gel electrophoresis (NEPHGE and SDS-PAGE) of detergent lysates from human B LCL Raji, intrinsically labeled with [^{35}S]methionine either in the presence of chloroquine (*CQ*), NH_4Cl, or control media (*CTRL*), and immunoprecipitated with mAb against Ia β (*Q5/13*) or γ chain (*VIC-Y1*). Orientation of the autoradiograms is with basic end to the left. Molecular masses (kilodaltons) are shown in the SDS-PAGE dimension. Control immunoprecipitations (not shown) were performed with murine myeloma P3X63Ag8 Ig. Known correspondences between NEPHGE spots and Ia subunits are summarized in the diagram (3, 17). The γ_2 and γ_3 spots represent alternate forms of γ chains associated with Ia oligomers, still of unclear significance (3). The multiple spots for α and β chains represent both polymorphic variants and metabolic precursors; *im* indicates biosynthetically immature proteins containing high-mannose core carbohydrates. Films were exposed for 15 d. Only the relevant parts of the NEPHGE gels is shown.

forms; the γ_s appear transiently in Ia oligomers, as conversion to two-chain oligomers takes place (5, 6, and V. Quaranta, unpublished observations); their metabolic fate is unknown. Since Q5/13 antibody immunoprecipitates Ia oligomers by binding to β chains (3), these patterns were consistent with γ chains dissociating from Ia oligomers concurrent to their own sialylation and to carbohydrate maturation on α and β subunits (6).

In parallel pulse-chase experiments, treatment of cells with chloroquine altered the dissociation of γ from α-β complexes. Although Q5/13 IP of chloroquine-treated cells were virtually indistinguishable from controls both at the 15 and 30 min time points, after 240 min, the pattern of chloroquine-treated cells was dramatically different from controls, as a striking increase in the intensity of γ_s chains was observed (Fig. 2, *Q5/13*). Note that the intensity of immature α, β, and γ chain spots, although somewhat higher than in the 240 min control, was lower than in the 30 min chloroquine panel (Fig. 2, *CQ*), indicating that chloro-

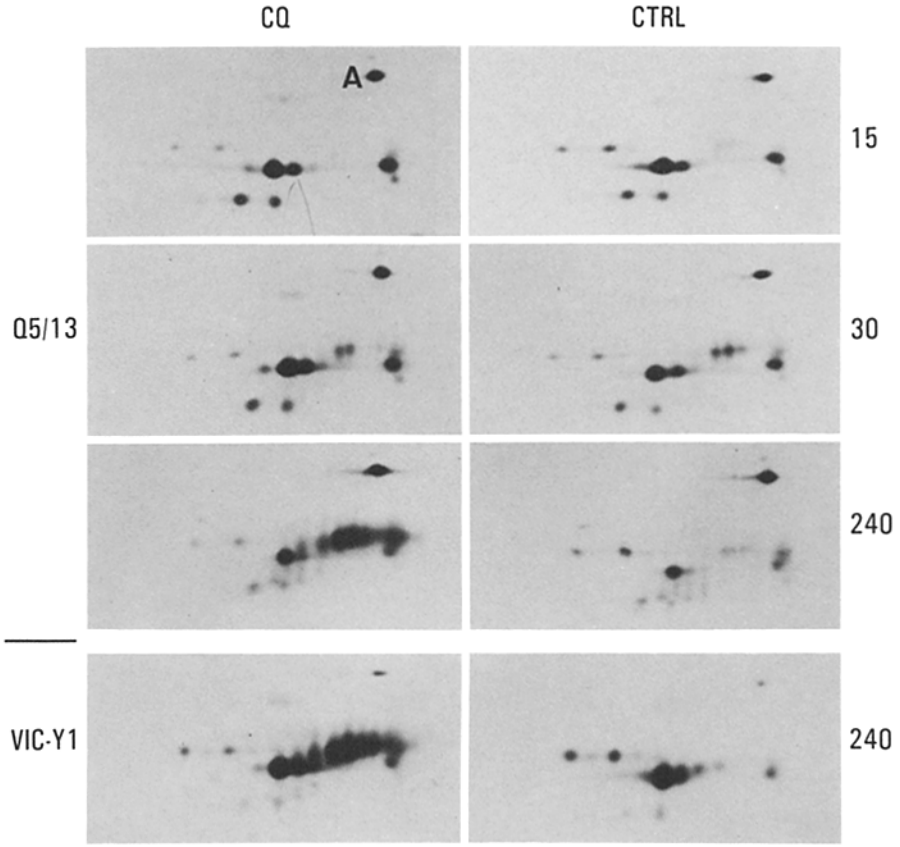

FIGURE 2. NEPHGE analyses of human B lymphoid cell line 721, pulse-labeled for 10 min, chased for times indicated (min) at the side of the panels, and immunoprecipitated with anti-γ (VIC-Y1) or -β (Q5/13) chain mAb. The series is a comparison of control (*CTRL*) versus chloroquine-treated (*CQ*) cells. Spots can be identified on the basis of the diagram in Fig. 1. Actin (*A*) is a commonly observed background spot; for unknown reasons, it is preferentially found in IP of certain mAb, such as Q5/13. Films were exposed for 5 d. Only the relevant part of the autoradiograms is shown.

quine greatly inhibited the dissociation of γ_s chains from Ia oligomers, but only slightly affected α, β, and γ chain maturation. In agreement, immunoprecipitation with anti-γ chain VIC-Y1 showed that, after 240 min, some fully processed forms of α and β chains remained associated to γ chains in treated, but not in control cells (Fig. 2, *VIC-Y1*).

Chloroquine did not exert an absolute block on γ chain dissociation. The relative content of mature α and β subunits (as estimated by intensity of spots) was consistently higher in anti-β then anti-γ IP, both at 240 min (Fig. 2), and more markedly at 16 h (Fig. 1). This suggested that, possibly because of compensatory mechanisms enacted by cells, Ia oligomers could escape chloroquine inhibition and dissociate into mature α-β dimers and γ chains as a function of time.

Within the limits of our experimental approaches, the results presented indicate that chloroquine selectively affected Ia biosynthesis in B cells by inhibiting

the step that dissociates the γ from the α and β subunits. The drug did not impair the rate of translation nor the initial processing steps of the Ia subunits. Later processing steps, involving accessibility to and functioning of Golgi glycosidases and glycosyltransferases, were also unaffected, since each Ia subunit was terminally glycosylated in the three-chain oligomers that accumulated in the presence of chloroquine.

In analogy with other chloroquine-sensitive intracellular transport pathways such as receptor-mediated endocytosis (7) and recycling of phosphomannosyl receptors (8), dissociation of γ chains from Ia oligomers may depend upon acidification of an endosome-like (10) compartment. This would explain its susceptibility to inhibition by chloroquine, whose principal effect on cells is the disruption of ATP-dependent membrane-associated proton pumps responsible for acidification of lysosomes and endosomes (10, 11). In agreement, two other agents that likewise block intravesicular acidification, NH_4Cl (7) and monensin (12), also affected γ chain dissociation (Fig. 1) (13). Monensin additionally blocked γ chain sialylation (13). However, since our data clearly showed that Ia oligomers containing γ_s may accumulate (Figs. 1 and 2), sialylation may contribute to, but is not sufficient for inducing γ chain dissociation, as previously proposed (13).

Chloroquine inhibits Ia-restricted antigen presentation in vitro, both in phagocytic (14, 15) and nonphagocytic (14–16) accessory cells. In most reports (14), such inhibition was explained by reduced antigen degradation by lysosomes. However, no biochemical analyses of Ia synthesis were performed (14–16). The possibility that such effects of chloroquine may be due in part to its interference with Ia biosynthesis should therefore be considered. It remains to be seen whether the postulated existence of acidic vesicles both in the inward/outward–bound pathways of antigen transport and in the biosynthetic pathway of Ia molecules may serve as a physical intracellular interface for coordinating the tasks of antigen presentation.

Summary

Biosynthetic conversion of Ia oligomers from three chains (α, β, γ) to two (α, β) before surface expression was inhibited in B lymphoid cells by treatment with chloroquine, resulting in the accumulation of Ia complexes composed of mature α and β chains, and γ chains at various states of sialylation. Other stages of Ia biosynthesis and processing appeared unaffected, indicating that chloroquine selectively interfered with the γ chain dissociating mechanism itself. Similar effects were also observed with ammonium chloride. Because of the nature of such lysosomotropic agents, these results suggest that an intracellular acidic compartment may be involved in processing Ia oligomers to accomplish dissociation from γ chains. Since chloroquine is known to inhibit Ia-restricted antigen presentation in accessory cells, our results raise the possibility that the pathways of antigen processing and Ia biosynthesis may use some common intracellular compartments.

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