Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 3029–3034, March 1999
Immunology

In vitro comparison of the antigen-binding and stability properties of the various molecular forms of IgA antibodies assembled and produced in CHO cells

JOSÉ BERDOZ*†, CORINNE TALLICHET BLANC*, MONIQUE REINHARDT*, JEAN-PIERRE KRAEHENBUHL*, AND BLAISE CORTHÉSY*‡§

*Swiss Institute for Experimental Cancer Research, Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland; and ‡Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Rue du Bugnon, CH-1011 Lausanne, Switzerland

Edited by John J. Mekalanos, Harvard Medical School, Boston, MA, and approved December 30, 1998 (received for review October 29, 1998)

ABSTRACT **The hallmark of a mucosal immune response is the production of antigen-specific secretory IgA (S-IgA) antibodies in external secretions. S-IgA consists of ten polypeptides produced in two different cell lineages. The heavy and light chains in plasma cells assemble into IgA, which on association with J chain become polymerized, whereas secretory component (SC) is added during transport across the epithelium. Recombinant chimeric mouse–human monomeric, dimeric, and S-IgA antibodies have been produced in a single CHO cell sequentially transfected with expression vectors carrying three independent selective markers for chimeric heavy and light chains, human J chain, and human SC, respectively. Biochemical characterization of the various molecular forms indicates that the assembly of the various polypeptides resulted in species of the expected size and covalence. All chimeric IgA antibodies retained the antigen-binding capacity of the parent mouse IgA antibody. The resistance of S-IgA to protease-rich intestinal washes was enhanced when compared with dimeric IgA lacking associated SC. Up to 20 μg of recombinant S-IgA per 1×10^6 cells were recovered in 24 h with the best producing clones. We conclude that CHO cells programmed *de novo* with four different genetic elements can assemble functional chimeric S-IgA.**

In humans, mucosal surfaces of the gut, the airways, and the urogenital tract are covered by epithelial layers that form tight barriers separating a rapidly changing external environment from a highly regulated internal compartment. To maintain the integrity of these vulnerable cellular barriers, mucosal surfaces have evolved specialized innate and adaptive defense mechanisms (1). Both cells and secretory IgA (S-IgA) antibodies contribute to adaptive specific mucosal immunity (2). S-IgA consists of at least two monomeric IgA units and two additional polypeptide chains, the J chain and SC (secretory component). The four polypeptides are produced by two distinct cell types. The heavy α chain, the light chain, and the J chain are synthesized and assembled into polymeric IgA in plasma cells, whereas SC, which corresponds to the ectoplasmic portion of the epithelial poly-Ig receptor, is added during transport of IgA across mucosal or glandular epithelia (3). In secretions, S-IgA bind antigen(s), thus preventing their adhesion to the luminal epithelial surface and facilitating their elimination by peristalsis or mucociliary movement. Intracellular neutralization of viruses occurs also during transcytosis of dimeric IgA (dIgA) across the epithelial cells (4).

IgA-mediated protection against viruses and bacteria has been demonstrated by administration of hybridoma-derived monoclonal IgA antibodies in mice (5–8). S-IgA antibodies assembled in plants afford specific protection in humans against oral streptococcal colonization for at least 4 months (9). Production of S-IgA has been obtained by using cocultures (10), *in vitro* combination of purified dIgA and SC (11, 12), or transfection of SC cDNA in IgA-expressing murine transfectomas (13). Because of their association with SC, S-IgA antibodies are well suited to resist acid denaturation and proteolytic degradation. We have shown that the presence of SC in S-IgA antibody delays degradation into $F(ab')_2$ fragments lacking the Fc effector function (14). This finding argues in favor of using the whole S-IgA molecule to maintain its integrity at mucosal surfaces. Clinical trials indicate that efficient mucosal protection requires milligram quantities of monoclonal IgA antibodies. Ideally, for large-scale production, S-IgA should be recovered from a single mammalian cell capable of intact glycosylation, should contain limited mouse sequences to avoid human anti-mouse antibody response, and should be able to activate human effector functions. So far, chimeric dIgA have been produced in myeloma cells expressing the murine J chain (15, 16).

We now report the production of high levels of antigen-specific chimeric mouse-human monomeric IgA (mIgA), dIgA, and S-IgA in CHO cells. Clones expressing up to 20 μg of the various molecular forms of IgA per 1×10^6 cells in 24 h have been obtained. Given that CHO cells secrete proteins with posttranslational modifications close to human cells, can be grown to high density in bioreactors, and that protein expression can be enhanced by using gene amplification, our approach makes possible the production of large quantities of S-IgA for clinical applications.

MATERIALS AND METHODS

Construction of Expression Vector pcDNA3:$V_H C\alpha 2m(1)$ and pcDNA3:$V_L C\kappa$. Expression vector pcDNA3:Cα2m(1) was constructed from pcDNA3 (Invitrogen) by insertion of the genomic regions either coding for human Cα2m(1) (17) or containing the mouse μ enhancer (18). The Cα2 region was amplified from cosmid Ig10 (19) by PCR with primers 5′-G-ACCC**AAGCTT**TCCAAC(C/T)GCAGGCC(C/T)(A/G)T-GG-3′ carrying the new site *Hin*dIII (bold) and 5′-ATAGT-TTA**GCGGCCGC**TTTCCCAAGTGCTGAGACC-3′ intro-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviations: CHO cells, Chinese hamster ovary cells; FCS, fetal calf serum; HRP, horseradish peroxidase; dIgA, dimeric IgA; mIgA, monomeric IgA; RSV respiratory syncytial virus; SC, secretory component; S-IgA, secretory IgA; SN, supernatant; TCA, trichloroacetic acid.

†Present address: Galli-Valerio Institute, Rue César-Roux 37, CH-1014 Lausanne, Switzerland.
§To whom reprint requests should be addressed at: Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Rue du Bugnon, CH-1011 Lausanne, Switzerland. e-mail: blaise.corthesy@chuv.hospvd.ch.

ducing the *Not*I site (bold). PCR cloning of the μ enhancer in plasmid pμ (20) was performed by using primers 5′-G**GAATTCCGCGGCCGC**GGTC(A/T)TTATTTTTAACC-3′ carrying the new sites *Eco*RI/*Not*I (bold) and 5′-CCC**AAGCTT**GGGATACATTTTAGAAGTCG-3′ introducing the *Hin*dIII site (bold). Expression vector pcDNA3:Cα2m(1) was assembled from a three-piece ligation including (*i*) plasmid pcDNA3 cleaved with *Eco*RI/*Not*I, (*ii*) the PCR fragment with the α2m(1) region treated with *Hin*dIII/*Not*I, and (*iii*) the μ enhancer-containing PCR fragment cut with *Eco*RI/*Hin*dIII. Expression vector pcDNA3:Cκ was constructed after pcDNA3 and comprises the genomic region coding for human Cκ and a genomic fragment containing the mouse κ enhancer (21). The Cκ region was amplified by PCR with primers 5′-CGC**GGATCC**GCGCCTGGAAGAGGCACAGGG-3′ carrying the new site *Bam*HI (bold) and 5′-ATAGTTTA**GCGGCCG**CCCCGTAAGACCTGTCACCC-3′ introducing the *Not*I site (bold) in combination with genomic DNA prepared from the human Raji lymphoma cell line (ATCC CCL86). To isolate the κ enhancer, primers 5′-G**GAATTCCGCGGCCGC**TTTTTATGTGTAAGACAC-3′ carrying the new sites *Eco*RI/*Not*I (bold) and 5′-CGC**GGATCC**GCGAATACTCTGATATTAGC-3′ introducing the *Bam*HI site (bold) were used in PCR together with plasmid pB1-14 (22). Expression vector pcDNA3:Cκ was assembled from a three-piece ligation including: Plasmid pcDNA3 cleaved with *Eco*RI/*Not*I, the PCR fragment with the κ region treated with *Bam*HI/*Not*I, and the κ enhancer-containing PCR fragment cut with *Eco*RI/*Bam*HI. Vectors pcDNA3:V_HCα2m(1) and pcDNA3:V_LCκ were obtained by ligation of rearranged genomic V_H and V_L regions from mouse hybridoma clone HNK20 (ATCC HB-11394) specific for respiratory syncytial virus (RSV) F glycoprotein (23) into pcDNA3:Cα2m(1) and pcDNA3:Cκ cut with *Kpn*I and *Not*I.

Construction of pcDNA3Hygro:J Chain. The region comprising the *neo*^{R} gene in pcDNA3 was excised by digestion with *Bst*BI and *Xma*I. A 1.1-kb fragment containing the *hyg*^{R} gene was amplified from plasmid pREP4 (Invitrogen) by using primers 5′-CGTGT**TCGAA**CCTTCCGTGTTTCAGTTAGCC-3′ containing a *Bst*BI site (bold) and 5′-TCCC**CCCGGG**AGCGTCAACAGCGTGCCGCC-3′ carrying a *Xma*I site (bold). After digestion with *Bst*BI and *Xma*I, the PCR fragment was introduced into the corresponding sites of pcDNA3, resulting in the production of pcDNA3Hygro. A 1.3-kb fragment comprising the cDNA for human J chain was amplified by PCR by using clone 49J (a 1.5-kb cDNA provided by E. Max, Bethesda, MD) and primers 5′-ACGC**GTCGAC**GCTCCAGTTTTTCAGAAG-3′ introducing a *Sal*I site (bold) and 5′-ATAGTTTA**GCGGCCGC**CTA*TTTATT*GCTCAGACTG-3′ introducing a *Not*I site (bold) and a concensus poly(A)$^+$ motif (italic). The PCR product was digested with *Sal*I, filled in with Klenow, then cut with *Not*I before ligation into pcDNA3Hygro treated with *Eco*RV and *Not*I, creating pcDNA3Hygro:Jchain.

Construction of pcDNA3His:SC. Excision of the region containing the *neo*^{R} gene in pcDNA3 was performed by cleaving the plasmid with *Bst*BI and *Xma*I. Plasmid pREP8 (Invitrogen) was used to amplify by PCR a 1.4-kbp fragment comprising the *his*^{R} gene. Primer 5′-CGTGT**TCGAA**GCTAAGTCAGCGACGCTG-3′ introduced the *Bst*BI restriction site (bold) and primer 5′-TCCC**CCCGGG**TGGAGGCCTAGGCTTTTGC-3′ introduced a *Xma*I site (bold). The resulting PCR product was treated with *Bst*BI and *Xma*I and cloned into *Bst*BI/*Xma*I-digested pcDNA3, leading to the production of pcDNA3His. A 1.85-kbp fragment carrying the cDNA for human SC was recovered by digesting plasmid pBS-hSC:End (11) with *Eco*RI and *Xba*I. The fragment comprising the SC cDNA was them ligated into *Eco*RI/*Xba*I-treated pcDNA3His, creating expression vector pcDNA3His:SC.

Establishment of Stable CHO Cells Expressing mIgA, dIgA, and S-IgA. The CHO dhfr$^-$ cells (CHO DUK$^-$) were obtained from the American Tissue Culture Collection (Manassas, VA; ATCC CRL 9096) and cultured in α-minimal essential medium supplemented with 10% dialyzed fetal calf serum (FCS) (HyClone), 10 mM Hepes (pH 7.0), and 50 μg/ml gentamycin. Cells (2×10^6) were electroporated (400 V; 250 μF; 2 pulses at an interval of 1 min) with 10 μg of *Pvu*I-linearized plasmids pcDNA3:V_HCα2m(1) and pcDNA3:V_LCκ. Selection was started 30–48 h later in the presence of G418 (500 μg/ml). Cell clones were isolated at one cell per well in 96-well culture dishes by using a cell sorter. Selection was continued for 2 more weeks, and emerging clones were then tested by ELISA (see below). Clone 22, which produced the highest amount of IgA2m(1), was amplified to serve as the recipient cell for subsequent transfection with *Avi*II-linearized pcDNA3Hygro:Jchain. Selection was carried out in the presence of G418 (500 μg/ml) and hygromycin (400 μg/ml). Clone F, which produced dIgA, was transfected with pcDNA3His:SC linearized with *Pvu*I. Selection was performed in the presence of 7 mM histidinol for 1 week, and in the presence of G418 (500 μg/ml), hygromycin (400 μg/ml), and 7 mM histidinol afterward. Of 12 clones analyzed, clone 6 was chosen for biochemical studies.

Anti-Human IgA ELISA. Rabbit anti-human κ-chain-specific antiserum (Dako) diluted 1:1,000 in 50 mM Na_2CO_3 buffer (pH 9.6) was used to coat wells (50 μl/well) of Nunc maxisorp immunoplates overnight at 4°C. The plates were blocked with 5% nonfat dry milk in PBS and 0.1% Tween 20 (PBS-T). Fifty microliters of IgA supernatant (SN) and human IgA standards (Sigma; range, 0.5–20 ng) were applied to the wells and incubated for 1 h at 37°C. Plates were washed three times with PBS-T, and IgA was detected with biotinylated rabbit anti-human IgA α-chain (Dako) diluted 1:1,000 in 0.5% nonfat dry milk in PBS-T. Bound antibodies were detected with streptavidin-horseradish peroxidase (HRP) conjugate diluted 1:1,000 in PBS-T and chemiluminescence (Amersham).

RSV Glycoprotein F-Specific ELISA. The antigen-binding capacity of the antibodies was detected by sandwich ELISA as above, except that a 1:1,000 dilution of a lysate of VERO cells infected with RSV (Oravax, Cambridge, MA) was used as the capture reagent.

Biochemical Analysis of Recombinant mIgA, dIgA, and S-IgA. *Immunoprecipitation.* Three milliliters of CHO cell SN was incubated with 6 μl of goat anti-human κ-chain-specific antiserum (Cappel) for 1 h at 23°C; 100 μl of protein A Sepharose slurry equilibrated in PBS were then added and the mixture was incubated overnight at 4°C. The antigen-antibody-beads pellet was washed three times with 1 ml of buffer consisting of 50 mM Tris·HCl (pH 7.0), 150 mM NaCl, 5 mM EDTA, and 1% Triton X-100. The beads were mixed with 1 volume of 2× SDS-PAGE sample buffer (24) containing or lacking 100 mM DTT, boiled for 3 min, and submitted to SDS/PAGE (25).

Immunoblotting. Proteins separated by SDS/PAGE were transferred to polyvinylidene difluoride membranes. Nonspecific binding sites were saturated for 30 min at 23°C by incubation with PBS-T/5% nonfat dry milk. The membrane was probed for 1 h at 23°C by using the following antisera/antibodies: biotinylated rabbit anti-human IgA α-chain-specific antiserum (Dako), working dilution: 1:1,000; rabbit anti-human κ-chain-specific antiserum (Dako), working dilution: 1:3,000; rabbit anti-human J chain-specific antiserum (26), working dilution: 1:1,000; mouse anti-human SC-specific mAb (Sigma), working dilution: 1:3,000. Bound antibodies were detected with HRP-conjugated streptavidin diluted 1:1,000; HRP-conjugated goat anti-rabbit IgG diluted 1:3,000 (Sigma); HRP-conjugated goat anti-mouse IgG diluted 1:3,000 (Sigma), in combination with the chemiluminescence assay (Amersham).

Digestion of Recombinant IgA/S-IgA with Intestinal Washes. Mouse intestinal washes were obtained as reported in Crottet and Corthésy (14). Before mixing with intestinal

washes, CHO cell SN were filtered onto a 100-kDa Centriprep unit (Amicon) to delete serum protein that inhibited digestion. Digestion was performed at 37°C for 16 h with filtered SN containing 2 μg of IgA and various dilutions of intestinal washes. Digestion products were analyzed by immunoblotting as described above.

RESULTS AND DISCUSSION

Construction of S-IgA Expression Vectors and Transfection of CHO Cells. Expression cassettes allowing production of chimeric IgA, J chain, and SC are diagrammed in Fig. 1. Based on the work of Page and Sydenham (27), a single marker was used to select cells expressing IgA heavy and light chain, whereas expression of J chain and SC was selected independently in two subsequent steps to facilitate the successive isolation of CHO clones expressing high levels of mIgA, dIgA, and S-IgA. In addition to intron μ and Cκ enhancers (28), the heavy and light chain vectors contained intron and 3′-untranslated regions that are known to improve expression of light chain in COS and CHO cells (29). The cell line CHO DUK$^-$ was cotransfected with pcDNA3:V_HCα2m(1) and pcDNA3:V_LCκ expression vectors containing the murine variable regions of the HNK-20 mAb (23). Transfectants were selected in the presence of 500 μg/ml G418 for 2 weeks. The best expresser (clone 22) was subjected to extensive biochemical characterization, including the determination of the degree of polymerization and covalent assembly of the different subunits, the retention of antigen-binding capacity, and the resistance to proteolysis (see below). Clone 22 was subsequently transfected with pcDNA3Hygro:Jchain vector and cells were selected for 2 weeks in the presence of both G418 and hygromycin B. Twenty double-resistant colonies were further analyzed, and one clone (F) producing dIgA-containing J chain was transfected with pcDNA3His:SC, resulting in the generation of clone 6 producing S-IgA.

CHO Cells Assemble and Produce Chimeric mIgA, dIgA, and S-IgA. Most of the IgA produced by clone 22 cells was monomeric as revealed by immunodetection with anti-α-chain antiserum of trichloroacetic acid (TCA)-concentrated SN resolved by SDS/PAGE under nonreducing conditions (Fig. 2*A*, lane 1). Although IgA2m(1) antibody is known to lack covalent disulfide bonds between the heavy and light chains (30), we also recovered minor covalent HL and H_2L_2 forms. When SN were concentrated by immunoprecipitation with anti-κ-chain antiserum rather than TCA, bands of identical intensity and size were observed, indicating that the light chain is preferentially associated with the heavy chain to form noncovalent, stable H_2L_2 complexes that can be quantitatively precipitated (Fig. 2*A*, lane 4).

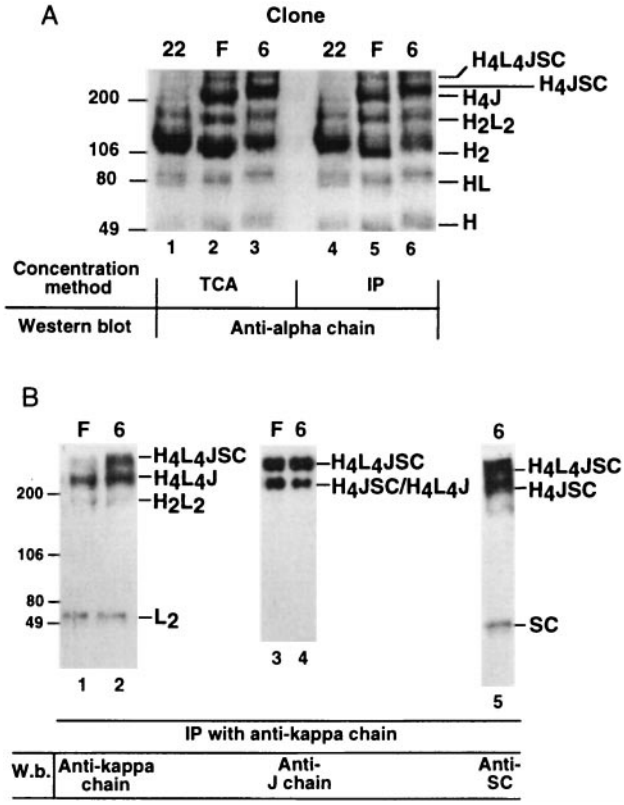

FIG. 2. Polymeric IgA molecules assemble in CHO cells. *(A)* Cell culture SN from clones 22, F, and 6 were concentrated by precipitation with TCA, or immunoprecipitation (IP) with anti-κ-chain antiserum/protein A Sepharose beads. After separation onto a 5–13% polyacrylamide gradient gel in SDS under nonreducing conditions, the proteins were transferred onto membranes and detected by using an anti-α-chain antiserum. Molecular weight markers run in parallel served to identify the various polymeric forms marked as combination of H (heavy), L (light), and J (J) chains and SC (SC). *(B)* Cell culture SN from clones F and 6 were concentrated with anti-κ-chain antiserum/protein A Sepharose beads and processed as in *A*. Blotted proteins were incubated with antisera directed against the κ-chain (lanes 1 and 2), the J chain (lanes 3 and 4), and SC (lane 5) to confirm the nature of species identified in *A*. Note that each specific antiserum has a distinct sensitivity, which explains the different relative intensity of the multiple molecular forms.

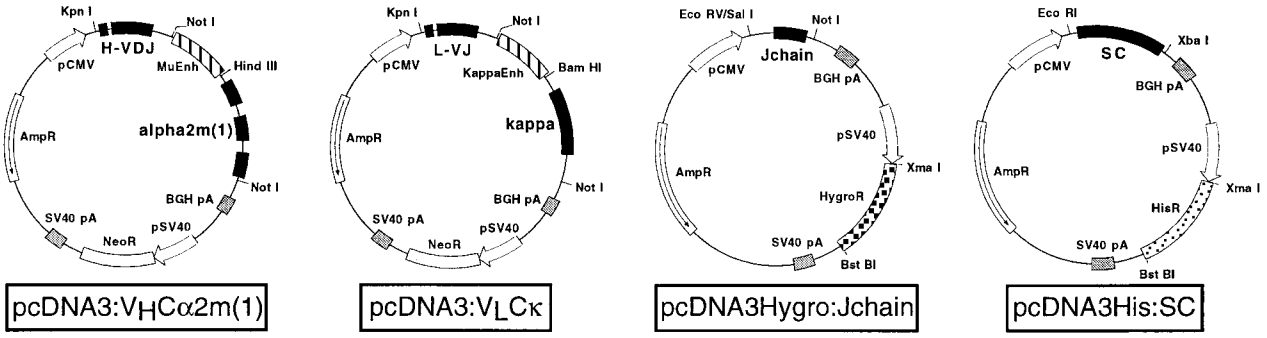

FIG. 1. Schematic representation of the expression vectors used in this study. All constructs were derived from pcDNA3. Plasmids pcDNA3:V_HCα2m(1) and pcDNA3:V_LCκ contain the original ORF for neomycin resistance (neo^R), pcDNA3Hygro:Jchain the ORF for hygromycin B resistance (hygro^R), and pcDNA3His:SC the ORF for histidinol resistance (his^R). The two black boxes called H-VDJ and L-VJ represent the genomic DNA coding for the murine heavy and light variable regions isolated from HNK-20 hybridoma. The other black boxes contain the human genomic region comprising the three α-chain domains, the human genomic region coding for the κ-chain, and the human cDNAs for the J chain and SC, respectively. Striped segments span the μ and κ enhancers obtained from mouse genomic fragments. The restriction sites used for the construction of the vectors are indicated.

IgA produced by clone F transfected with J chain cDNA were similarly analyzed. When compared with clone 22, two additional species corresponding to dIgA (H_4J and H_4L_4J) were detected on immunoblots (Fig. 2*A*, lanes 2 and 5). Other forms (H_2L_2, HL, H_2, H) produced by clone 22 remained unassociated with J chain, indicating that J chain-mediated dimerization was probably rate-limiting (Fig. 3). In this respect, CHO cells assemble and polymerize IgA to the same extent as most IgA-secreting hybridomas (20–80% dIgA secreted). Because a reliable quantitative ELISA for human J chain is not available, and because unpolymerized J chain is rapidly degraded, we could not establish whether the partial polymerization we measured was a consequence of a low J chain production.

Clone 6 produced S-IgA molecules that showed an immunoblot pattern similar to that of clone F. The upper H_4J band was shifted to a slower migrating band corresponding to H_4JSC complexes (Fig. 2*A*, lanes 3 and 6), indicating that SC associates covalently with dIgA as in human colostral milk (31). The detection of a band close to the running-stacking gel interface indicates that some light chain was covalently bound

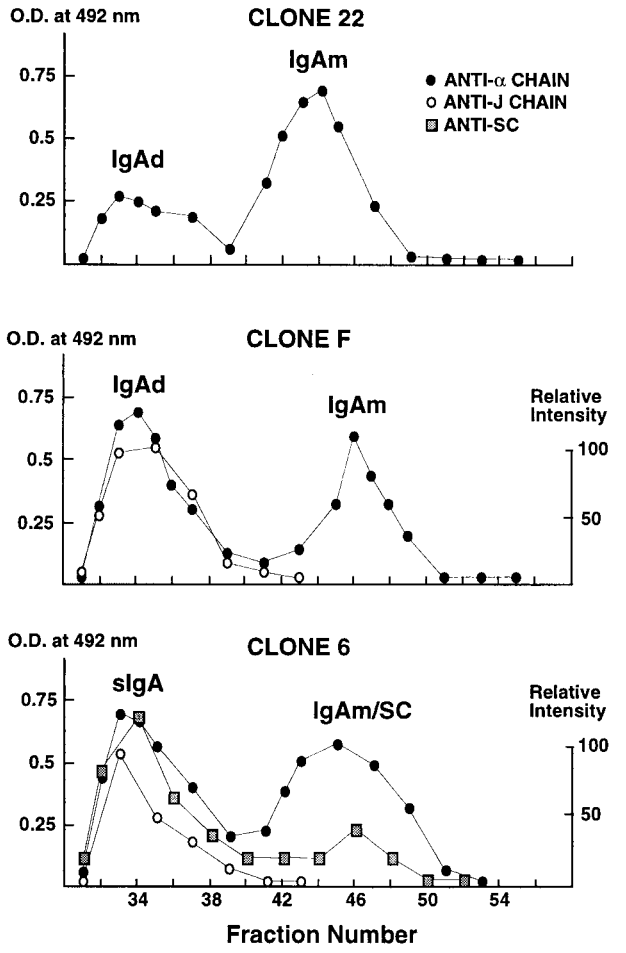

FIG. 3. Analysis of the composition of IgA forms secreted by clones 22, F, and 6. Two hundred microliters of crude SN was separated by chromatography on a Superose 12 column (Pharmacia) equilibrated and run in PBS. Fractions of 250 μl were collected and analyzed by ELISA (α-chain and SC) or Western blot (J chain). For ELISA, the resulting OD at 492 nm is plotted. For Western blot analysis, half of the fraction was concentrated by TCA precipitation before loading on a 15% polyacrylamide/SDS gel. After transfer, the protein was detected with rabbit anti-J chain antiserum, followed by mouse anti-rabbit antibody conjugated with HRP and chemiluminescence. Films were scanned by using an Elscript 400-AT/SM densitometer, and values were plotted with the strongest intensity arbitrarily fixed as 100%.

with the heavy chain (Fig. 2*B* and data below). The dIgA/mIgA ratio in clone 6 was much higher than that estimated for clone F, yet the signals corresponding to H_4J and H_4JSC complexes were comparable. Presumably, because clone 6 was screened on the basis of SC level in SN, and assuming that optimal secretion of SC was mediated after intracellular binding to dIgA carrying the J chain (13), our mode of selection favored the isolation of a clone expressing S-IgA. The presence of positive signals with anti-κ, anti-J chain, and anti-SC antisera in high M_r species (H_4L_4J and H_4L_4JSC) confirms the covalent association of light chains, J chain, and SC with heavy chains (Fig. 2*B*, lanes 1–5). Our results indicate that genetically engineered CHO cells are able to efficiently assemble 10 polypeptides and secrete them as S-IgA antibodies.

The immunoblot analysis performed above does not indicate whether inactive aggregates form during production or whether artifactual structures might arise from biosynthesis in CHO cells. To assess the proportion of properly assembled complexes in solution, the culture media from clones 22, F, and 6 were chromatographed onto a Superose 12 column equilibrated with PBS. Two peaks corresponding to dIgA/S-IgA and mIgA were resolved on the column irrespective of the clone (Fig. 3). Clone 22 produced a significant proportion of dIgA that could not be revealed by the analysis performed in Fig. 2. Half of the IgA secreted by clone F was monomeric, with J chain being strictly observed in the dIgA peak. In the SN of clone 6, J chain eluted in association with S-IgA, and most of SC coeluted with dIgA, yet some free SC remained detectable. Transfection of an IgA-secreting myeloma clone with a SC construct similarly resulted in the detection of free SC (13).

The cellular machinery of CHO cells can synthesize, assemble, and secrete the products of exogenous genetic elements given either under the form of genomic DNA or cDNA. Cells stably transfected with the heavy and light chain constructs produced mainly mIgA, although some oligomers were detected under nondenaturing conditions, a property associated with the α-chain tail piece (32). This is consistent with the observation that colostral milk IgA lacking the J chain is also produced as oligomers (33). J chain assembles with dIgA and was never found bound with a nonpolymeric molecular form. This finding suggests that the information for proper assembly between the α and J chains is provided by the polypeptides themselves, and not by cell-specific enzymatic activity(ies). S-IgA produced by CHO cells contain essentially covalently linked SC.

Factors Affecting S-IgA Production by CHO Cells. Clones 22, F, and 6 grown to confluence were cultured for 24, 48, and 96 h in fresh FCS-supplemented medium, and antibody secretion was measured by ELISA and normalized to a fixed number of live cells. All three clones expressed $\approx$20 μg per 1×10^6 cells during the first 24 h (Fig. 4*A*). The recovery increased 2.5-fold during the next 24 h, and further incubation for up to 96 h yielded 20–30% more antibody as compared with 48 h. These high levels of expression were achieved in the absence of methotrexate amplification, and fall in the range reported for IgG production by stably transfected CHO cells (29). Similar yields were reported for murine hybridoma clones (34), indicating that intron regulatory elements do not adversely affect expression in nonlymphoid CHO cells. Gene amplification (35), however, might not favor better expression, as we have shown that mammalian cell lines cannot process nascent glycoprotein beyond a certain limit (36). We have observed that FCS is required for IgA antibody production. No recombinant protein was recovered from clone 6 cultivated in FCS-free conditions for up to 48 h (Fig. 4*B*, compare even and odd lanes). This result is likely caused by the fact that the original clone P2 was cultured in FCS-containing medium. Indeed, production of heterologous proteins in CHO cells does not require FCS provided the cells are adapted to FCS-free

A

CLONE \ TIME	24 hr	48 hr	96 hr
22 (IgAm)	22 μg	50 μg	61 μg
F (IgAd)	20 μg	48 μg	65 μg
6 (sIgA)	19 μg	55 μg	59 μg

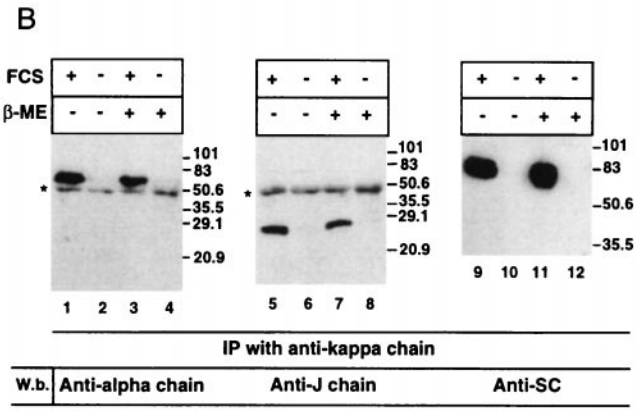

FIG. 4. Effect of time, FCS, and 2-mercaptoethanol on IgA expression. *(A)* Level of IgA production in SN of clones 22, F, and 6, expressed in μg per 1×10^6 cells as a function of time. IgA were measured by ELISA by using purified dIgA (clones 22 and F) and S-IgA (clone 6) as standards. *(B)* Effect of FCS and 2-mercaptoethanol (β-ME) on expression of S-IgA by clone 6. The cell culture SN was concentrated by immunoprecipitation (IP) with anti-κ-chain antiserum/protein A Sepharose beads, and separated by SDS/PAGE under reducing conditions. After transfer onto membranes, polypeptides were detected with antisera to the α-chain, the J chain, and SC. Molecular weight standards are indicated on the side of the gel. Asterisks indicate cross-reactive bands corresponding to the IgG heavy chain in the antiserum used for immunoprecipitation.

conditions (37). Addition of 2.5 mM 2-mercaptoethanol (final concentration) did not stimulate secretion whether FCS was present or not (Fig. 4*B*), in contrast to what was shown for IgM, another polymeric Ig (29).

Antigen Binding Capacity and *in Vitro* Stability of Recombinant Chimeric S-IgA Antibodies. The antigen-binding capacity of the three clones (22, F, and 6) was measured by ELISA by using, as coating, a lysate of Vero cells infected with a RSV strain expressing the glycoprotein F antigen. The amount of IgA in each SN was adjusted to 20 μg/ml, and binding to the antigen of antibody dilutions was assessed by detection with anti-α-chain antiserum. All three CHO lines expressing antibody light and heavy chains assembled functional antibodies (Fig. 5*A*). J chain-mediated dimerization or the presence of SC in the IgA molecule did not alter its antigen-binding capacity, which could be detected with as little as 10 ng/ml of antibody.

S-IgA assembled *in vitro* with recombinant SC and purified dIgA were shown to be more resistant to intestinal proteases than purified dIgA alone (14). To further characterize dIgA and S-IgA secreted by CHO clones, we compared their respective sensitivity to protease exposure. Degradation of the α-chain (62 kDa) on incubation at 37°C for 16 h with dilutions of protease-containing intestinal washes into fast migrating species (36–40 kDa) reflects the conversion of intact IgA to $F(ab')_2$ (Fig. 5*B* and ref. 14). Ten times more proteases were required to digest S-IgA (clone 6) than dIgA (clone F) (Fig. 5*B*, compare lanes 2 and 7). The protective effect of SC was detectable despite the presence of uncomplexed dIgA in the SN of clone 6 (Fig. 5*B*), and explains why some degradation occurred at the lowest concentration of intestinal proteases.

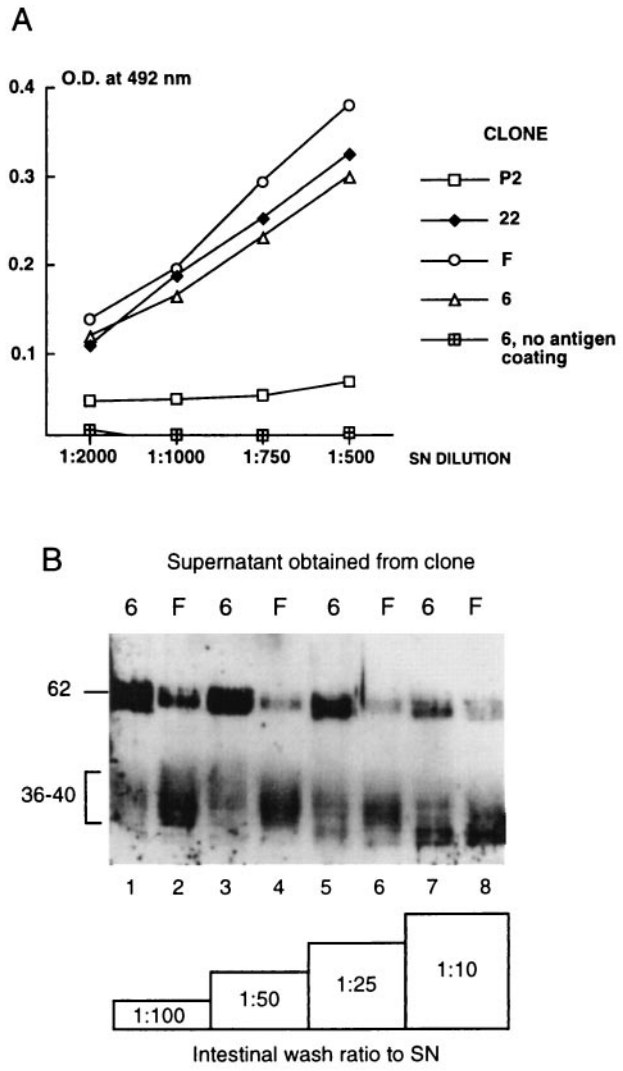

FIG. 5. Antigen-binding activity and comparative digestion patterns of IgA molecular forms secreted by CHO cells. *(A)* Crude CHO SN were incubated in wells coated with RSV whole lysate containing the glycoprotein F antigen. The bound antibodies were detected with biotinylated antibodies to the α-chain, followed by streptavidin conjugated with HRP. The initial concentration of each antibody solution obtained from clones 22, F, and 6 was standardized to 20 μg/ml by diluting the SN with cell culture medium. Results are means of three separate experiments done in duplicates. SN of untransfected CHO cell clone P2 and wells noncoated with the antigen were used as controls. Lower dilutions of the SNs resulted in loss of linearity of the response curve. *(B)* FCS-free SN containing identical amounts of recombinant IgA were combined with four different dilutions of pooled intestinal washes, resulting in an antibody/intestinal wash ratio ranging from 1:100 to 1:10. Digestion was carried out for 16 h at 37°C. Degradation products were separated by SDS/PAGE under reducing conditions and assessed by immunodetection of the α-chain. The position of the intact α-chain lies at 62 kDa, whereas degradation produces a series of bands at 36–40 kDa (14).

Functional characterization of recombinant IgA expressed in CHO cells allowed us to demonstrate biochemical properties similar or identical to native or hybridoma-derived molecules. The antigen-binding capacity of IgA antibodies produced in CHO cells was comparable to that of mouse hybridoma antibodies (data not shown), and dimerization with J chain or addition of SC did not alter the antigen-binding activity. This result is in agreement with the binding properties of SC-dIgA reconstituted *in vitro* (12). The presence of SC in S-IgA expressed by clone 6 enhanced protease resistance as compared with dIgA produced by clone F. Sensitivity to

proteases, normal secretion, and proper assembly are IgA characteristics dependent on sugar integrity (14, 38). CHO cells express glycosyl transferases that generate *N*- and *O*-linked glycosylation patterns similar to those produced by human cells (39). S-IgA expressed in CHO cells exhibit features which make them resemble closely their human counterpart and might thus favor their efficacy.

Our work presents a demonstration that large quantities of an active S-IgA antibody molecule can be assembled in a single, heterologous mammalian cell programmed with four exogenously delivered constructs. The strategy we followed has numerous advantages: (*i*) Fv DNAs isolated from pathogen-specific hybridomas or phage single chain can easily be converted into the very stable IgA isotype; (*ii*) S-IgA antibodies can be efficiently produced in one step; (*iii*) the functional properties of the various molecular forms of IgA with the same Fab can be compared; and (*iv*) the effector function of IgA antibodies of different isotype can be compared. In addition, comparison with IgA/S-IgA produced in plants might also help unraveling structure–function relationship in SC.

Production of sufficient amounts of IgA/S-IgA antibodies of defined specificity makes it possible to study how S-IgA interferes with bacterial and viral adhesion, and how the antibody affects pathogen-mediated signal transduction in *in vivo*/*in vitro* established models (4, 40). In addition, it brings IgA/S-IgA antibodies one step closer to application as recombinant therapeutic proteins. Mucosal administration of IgA for passive protection (41) or as a topical mucosal vaccine vehicle for otherwise unstable T and B epitope peptides (42) will certainly benefit from such an expression system.

We thank Dr. E. Max for providing human J chain cDNA, Dr. C. Kaetzel for human SC cDNA, and Dr. M.-P. Lefranc for human heavy and light chain genomic DNA. We acknowledge Dr. I. Corthésy-Theulaz for preparing the murine intestinal washes. We thank Drs. L. Hathaway and S. Hopkins for critical reading of the manuscript. This work was supported by grants from the Swiss National Science Foundation to J.P.K. (SNSF 31-47110.96 and 31-47296.96) and B.C. (SNSF 3100-050912.97).

1. Neutra, M. R. (1999) *Curr. Top. Microbiol. Immunol.* **236,** 17–32.
2. Corthésy, B. & Kraehenbuhl, J.-P. (1999) *Curr. Top. Microbiol. Immunol.* **236,** 93–111.
3. Mostov, K. E. (1994) *Annu. Rev. Immunol.* **12,** 63–84.
4. Mazanec, M. B., Nedrud, J. G., Kaetzel, C. S. & Lamm, M. E. (1993) *Immunol. Today* **14,** 430–435.
5. Michetti, P., Mahan, M. J., Slauch, J. M., Mekalanos, J. J. & Neutra, M. R. (1992) *Infect. Immun.* **60,** 1786–1792.
6. Lee, C. K., Weltzin, R., Soman, G., Georgakopoulos, K. M., Houle, D. M. & Monath, T. P. (1994) *Infect. Immun.* **62,** 887–891.
7. Weltzin, R., Traina-Dorge, V., Soike, K., Zhang, J. Y., Mack, P., Soman, G., Drabik, G. & Monath, T. P. (1996) *J. Infect. Dis.* **174,** 256–261.
8. Ruggeri, F. M., Johansen, K., Basile, G., Kraehenbuhl, J.-P. & Svensson, L. (1998) *J. Virol.* **72,** 2708–2714.
9. Ma, J. K. C., Hikmat, B. Y., Wycoff, K., Vine, N. D., Chargelegue, D., Yu, L., Hein, M. B. & Lehner, T. (1998) *Nat. Med.* **4,** 601–606.
10. Hirt, R. P., Hughes, G. J., Frutiger, S., Michetti, P., Perregaux, C., Jeanguenat, N., Neutra, M. R. & Kraehenbuhl, J.-P. (1993) *Cell* **74,** 245–255.
11. Rindisbacher, L., Cottet, S., Wittek, R., Kraehenbuhl, J.-P. & Corthésy, B. (1995) *J. Biol. Chem.* **270,** 14220–14228.
12. Lüllau, E., Heyse, S., Vogel, H., Marison, I., von Stockar, U., Kraehenbuhl, J.-P. & Corthésy, B. (1996) *J. Biol. Chem.* **271,** 16300–16309.
13. Chintalacharuvu, K. R. & Morrison, S. L. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6364–6368.
14. Crottet, P. & Corthésy, B. (1998) *J. Immunol.* **161,** 5445–5453.
15. Atkin, J. D., Pleass, R. J., Owens, R. J. & Woof, J. M. (1996) *J. Immunol.* **157,** 156–159.
16. Chintalacharuvu, K. R. & Morrison, S. L. (1996) *J. Immunol.* **157,** 3443–3449.
17. Flanagan, J. G., Lefranc, M. P. & Rabbitts, T. H. (1984) *Cell* **36,** 681–688.
18. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. (1983) *Cell* **33,** 717–728.
19. Flanagan, J. G. & Rabbitts, T. H. (1982) *Nature (London)* **300,** 709–713.
20. Grosschedl, R. & Baltimore, D. (1985) *Cell* **41,** 885–897.
21. Emorine, L. J., Marullo, S., Delavier-Klutchko, C., Kaveri, S. V., Durieu-Trautmann, O. & Strosberg, A. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6995–6999.
22. Brinster, R. L., Ritchie, K. A., Hammer, R. E., O'Brien, R. L., Arp, B. & Storb, U. (1983) *Nature (London)* **306,** 332–336.
23. Berdoz, J., Monath, T. P. & Kraehenbuhl, J.-P. (1995) *PCR Methods Appl.* **4,** 256–264.
24. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
25. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
26. Hendrickson, B. A., Conner, D. A., Ladd, D. J., Kendall, D., Casanova, J. E., Corthésy, B., Max, E. E., Neutra, M. R., Seidman, C. E. & Seidman, J. G. (1995) *J. Exp. Med.* **182,** 1905–1911.
27. Page, M. J. & Sydenham, M. A. (1991) *Bio/Technology* **9,** 64–68.
28. Emorine, L., Kuehl, M., Weir, L., Leder, P. & Max, E. E. (1983) *Nature (London)* **304,** 447–449.
29. Fouser, L. A., Swanberg, S. L., Lin, B. Y., Benedict, M., Kelleher, K., Cumming, D. A. & Riedel, G. E. (1992) *Bio/Technology* **10,** 1121–1127.
30. Jerry, L. M., Kunkel, H. & Grey, H. M. (1970) *Proc. Natl. Acad. Sci. USA* **65,** 557–563.
31. Kerr, M. A. (1990) *Biochem. J.* **271,** 285–296.
32. Morton, H. C., Atkin, J. D., Owens, R. J. & Woof, J. M. (1996) *J. Immunol.* **157,** 156–159.
33. Tomasi, T. B. J. & Czerwinski, D. S. (1976) *Scand. J. Immunol.* **5,** 647–653.
34. Harlow, F. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
35. Wood, C. R., Dorner, A. J., Morris, G. E., Alderman, E. M., Wilson, D., O'Hara, R. M., Jr., & Kaufman, R. J. (1990) *J. Immunol.* **145,** 3011–3016.
36. Cottet, S. & Corthésy, B. (1997) *Eur. J. Biochem.* **246,** 23–31.
37. Trill, J. J., Shatzman, A. R. & Ganguly, S. (1995) *Curr. Opin. Biotechnol.* **6,** 553–560.
38. Taylor, A. K. & Wall, R. (1988) *Mol. Cell. Biol.* **8,** 4197–4203.
39. Parekh, R. B., Dwek, R. A., Rudd, P. M., Thomas, J. R. Rademacher, T. W., Warren, T., Wun, T. C., Hebert, B., Reitz, B., Palmier, M. *et al.* (1989) *Biochemistry* **28,** 7670–7679.
40. Kerneis, S., Bogdanova, A, Kraehenbuhl, J.-P. & Pringault, E. (1997) *Science* **277,** 949–952.
41. Corthésy, B. (1997) *Biochem. Soc. Trans.* **25,** 471–475.
42. Corthésy, B., Kaufmann, M., Phalipon, A., Peitsch, M., Neutra, M. & Kraehenbuhl, J.-P. (1996) *J. Biol. Chem.* **271,** 33670–33677.