Oral administration of rabbit secretory IgA (sIgA) to adult BALB/c mice induced IgA+, IgM+, and IgG+ lymphoblasts and resulted in hybridomas producing IgA, IgM, and IgG1 antibodies to the secretory component (SC). This suggests that SC could serve as a vector to target protective epitopes into mucosal lymphoid tissue and elicit an immune response. We tested this concept by inserting a Shigella flexneri invasin B epitope into SC, which, following reassociation with IgA, was delivered orally to mice. To identify potential insertion sites at the surface of SC, we constructed a molecular model of the first and second Ig-like domains of rabbit SC. A surface epitope recognized by an SC-specific antibody was mapped to the loop connecting the E and F strands of domain I. This 8-amino acid sequence was replaced by a 9-amino acid linear epitope from S. flexneri invasin B. We found that cellular trafficking of recombinant SC produced in mammalian CV-1 cells was drastically altered and resulted in a 50-fold lower rate of secretion. However, purification of chimeric SC could be achieved by Ni²⁺-chelate affinity chromatography. Both wild-type and chimeric SC bound to dimeric IgA, but not to monomeric IgA. Reconstituted sIgA carrying the invasin B epitope within the SC moiety triggers the appearance of seric and salivary invasin B-specific antibodies. Thus, neo-antigenized sIgA can serve as a mucosal vaccine delivery system inducing systemic and mucosal immune responses.

The protection of the mucosal surfaces of the digestive, respiratory, and urogenital tracts is in part mediated by secretory IgA (sIgA). This antibody consists of IgA dimers associated with the J chain, which is acquired during the process of polymerization in plasma cells just before secretion, and with the secretory component (SC). SC is derived from the polymeric immunoglobulin receptor (pIgR), which binds and transports polymeric immunoglobulins across mucosal and glandular epithelia. Proteolytic cleavage of the extracellular portion of the receptor releases SC together with the bound IgA into mucosal secretions (1). SC belongs to the immunoglobulin superfamily of proteins and consists of a series of five Ig-like domains corresponding to the ectoplasmic portion of the poly-Ig receptor (2–5).

In addition to the protection of mucosal surfaces by cross-linking pathogens and promoting their clearance by peristalsis or mucociliary movement, sIgA in the intestinal lumen also selectively adheres to M cells. This was first observed in suckling rabbits as a local accumulation of milk sIgA on M cells of Peyer's patches (6). Subsequently, monoclonal mouse IgA, polyclonal rat IgA, and polyclonal IgG antibodies, radiolabeled or coupled to colloidal gold, were found to bind specifically to rabbit or mouse M cells and to compete with each other for binding sites (7). Selective sIgA binding and transport by M cells may play a role in the regulation of the mucosal immune response, but that role has not yet been defined. Thus, while sIgA would generally prevent the contact of antigens with mucosal surfaces, it could also promote re-uptake of small amounts of antigen by M cells.

The fact that orally administered sIgAs are efficiently transported by M cells in Peyer's patches raises the possibility that sIgA itself could serve as a vaccine delivery vector to target foreign epitopes into mucosa-associated lymphoid tissue. However, this would require that the foreign epitope be inserted without affecting the molecular folding of SC or the assembly and the function of sIgA. Also, the site of insertion must be surface-exposed, and the inserted epitope must be immunogenic.

The purpose of this study was to evaluate the feasibility of such an approach. We selected rabbit SC for epitope insertion because 1) the cDNA for the SC portion of pIgR was available (4), 2) SC was shown not to perturb the binding of sIgA to the antigen (8), 3) SC and IgA, can combine to form sIgA in vitro (9), and 4) a battery of monoclonal and polyclonal antibodies to SC is available to map the possible effects of epitope substitution on protein structure (see Table I). Our results first show a so far not identified function of domain I in the process of SC secretion, which nonetheless did not preclude either overexpression or IgA binding of chimeric SC. Second, oral immunization with “antigenized” sIgA elicits both a systemic and mucosal response against the inserted epitope, thereby suggesting that recombinant SC/IgA complexes could serve as a mucosal vaccine delivery system.
Antiserum/antibody | Target | Detection | References
--- | --- | --- | ---
Goat pAb 982 | SC, plgR, slgA | ELISA, IP, Western | Solari et al., 1985 (42)
Rabbit pAb anti-161 allele | SC, slgA | Western | Hanly et al., 1987
Guinea pig pAb | SC domains II and III | Western | Schaerer et al., 1990
Mouse mAb 303 | SC domain I, slgA | IP, Western | Kuhn et al., 1983
Mouse mAb 166 | plgR cysteolytic tail | IP, Western | Kuhn et al., 1983
Mouse mAb H10 | invasinB KDRTLIEQK epitope | IP, Western | Barzu et al., 1993

Abbreviations: pAb, polyclonal antisera; mAb, monoclonal antibody; IP, immunoprecipitation; ELISA, enzyme-linked immunosorbent assay; K, lysine; D, aspartic acid; R, arginine; T, threonine; L, leucine; I, isoleucine; E, glutamic acid; Q, glutamine.

**EXPERIMENTAL PROCEDURES**

**Monoclonal Antibodies (mAb) and Polyclonal Antisera (pAb)**

The mAb and pAb used in this study and their properties are listed in Table I.

**Immunodetection of Wild-type and Recombinant SC**

**Immunoprecipitation**—Cell extracts from biosynthetically labeled MDCK cells (10) were prepared in 3% SDS (final concentration) and diluted 10-fold in immunoprecipitation buffer (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 100 mM NaCl, 1% Triton X-100). Immunoprecipitation was performed using the relevant pAb in combination with protein A-Sepharose (Pharmacia Biotech, Inc.) or the appropriate mAb chemically coupled to Sepharose beads according to standard procedures (11).

**Western Blot Analysis**—IgA, SC, or plgR immunoprecipitated with the appropriate antibody (Table I) were separated by SDS-PAGE prior to transfer onto blotting membranes. plgR was detected using mAb 166 (1:1,000 dilution), whereas SC was detected with the guinea pig anti-serum (1:500). Primary antibody binding was detected using horseradish peroxidase-conjugated secondary antibodies (1:3,000) and the chemiluminescence detection reagent (Amersham Life Sciences).

**Enzyme-linked Immunosorbent Assay (ELISA)**—The concentrations of purified SC and IgA/IgG concentrations were measured as described in Rindisbacher et al. (9) and Lullau et al. (8), respectively. Standard curves for IgA were obtained using purified IgA from ascitic serum (1:500).

**Secretory IgA as a Vaccine Delivery System**

**Construction of Chimerized SC cDNA**—

1. **Oligonucleotides**

   - Forward primer: 5'-TACGCTAGTCACAATCACCAC-3'
   - Reverse primer: 5'-AAAGAGGAGGCGGGCCCTGGTCGTAGA-3'(hybridizing sequence, 314–337). In order to facilitate subsequent ligation of PCR products, the 5′-primer comprised the unique NotI site at position 324, and the 3′-primer carried a 5′-end by centrifugation at 12000 g for 15 min, and purification of secreted recombinant SC proteins was performed by Ni2+-chelate affinity chromatography according to the procedure given in Rindisbacher et al. (9).

**Generation of Vaccinia Virus Recombinants**—Recombinant viruses vHSV1-Sc6xHis and vHSV1-SC(1pAb6xHis) were generated by homologous recombination into the TK gene. This resulted in the in-frame fusion of the 11K ATG to the second codon of the SC coding region. Addition of a c-terminal 6-histidine tag was carried out by annealing of oligonucleotides 5′-CTCATCATTACATCACATCATAAAAGATCTCGAGTGTAC-3′ (coding strand), and 5′-ACTGAGATCTTATGATGTGATGATGATGAGTGATC-3′, followed by insertion into the KpnI site located 3′-upstream of the NotI site of pHSV1-SC. The sequence encoded 6 histidine residues, a translational termination codon, as well as XhoI and BglII sites to determine the orientation. The resulting plasmid was designated pHSV1-SC6xHis.

**Large Scale Production and Purification of Recombinant Rabbit SC**

**Antiserum and monoclonal antibodies used in this study**

**Plasmid pSC-11** was digested with NotI and BglII at position 888, and a 128- and 469-bp PCR product was synthesized with a 5′-end of the TK gene of constructs pHGS1-SC and pHGS1-SC(IpaB). Integration of the sc sequences into pHSV1-SC(IpaB) was achieved by annealing of the 128- and 469-bp PCR products with a 5′-end of the TK gene. This resulted in the in-frame fusion of the 11K ATG to the second codon of the SC coding region. Addition of a c-terminal 6-histidine tag was carried out by annealing of oligonucleotides 5′-CTCATCATTACATCACATCATAAAAGATCTCGAGTGTAC-3′ (coding strand), and 5′-ACTGAGATCTTATGATGTGATGATGATGAGTGATC-3′, followed by insertion into the KpnI site located 3′-upstream of the NotI site of pHSV1-SC. The sequence encoded 6 histidine residues, a translational termination codon, as well as XhoI and BglII sites to determine the orientation. The resulting plasmid was designated pHSV1-SC6xHis. Plasmid pHSV1-SC(1pAb6xHis) encoding antigenic SC was obtained by cleaving plasmid pHSV1-SC6xHis with NotI and BglII and replacing the fragment by the corresponding mutated fragment coming from plasmid pSC(1pAb).

**Concentration of Expression and Insertion Vectors**—Epipod insertion was performed by inserting the fragment NotI(324)/SalI(1834) recovered from pSC(1pAb) into pLkneo-P1R(15) digested with the same two enzymes. The resulting construct was called pLkneo-P1R(1pAb).

**Generation of Vaccinia Virus Recombinants**—Recombinant viruses vHSV1-Sc6xHis and vHSV1-SC(1pAb6xHis) were generated by homologous recombination into the TK gene. This resulted in the in-frame fusion of the 11K ATG to the second codon of the SC coding region. Addition of a c-terminal 6-histidine tag was carried out by annealing of oligonucleotides 5′-CTCATCATTACATCACATCATAAAAGATCTCGAGTGTAC-3′ (coding strand), and 5′-ACTGAGATCTTATGATGTGATGATGATGAGTGATC-3′, followed by insertion into the KpnI site located 3′-upstream of the NotI site of pHSV1-SC. The sequence encoded 6 histidine residues, a translational termination codon, as well as XhoI and BglII sites to determine the orientation. The resulting plasmid was designated pHSV1-SC6xHis. Plasmid pHSV1-SC(1pAb6xHis) encoding antigenic SC was obtained by cleaving plasmid pHSV1-SC6xHis with NotI and BglII and replacing the fragment by the corresponding mutated fragment coming from plasmid pSC(1pAb).

**Large Scale Production and Purification of Recombinant Rabbit SC**

**Construction of Chimerized SC cDNA**—A rabbit plgR cDNA fragment (161 bp) was recovered from plasmid pSC-11 (4) by HindIII/SalI digestion. The 1082-bp fragment served as a template for two PCRs aimed at the insertion of two unique restriction sites for subsequent sequence replacement within domain I of the rabbit SC clone. In the first reaction, an AccI site (bold characters) was created at position 418 using oligonucleotides 5′-AGGAGGAGGCGGGCCCTGGTCGTAGA-3′ and 5′-AACCTCCCTTTTTCAGGGAAGTGC-3′ (hybridizing sequence, 429–394)
secretory IgA as a Vaccine Delivery System

Fig. 1. Characterization of monoclonal antibody 36.4. Samples of rabbit whey were run on a 5% polyacrylamide gel and analyzed as follows: staining with Coomassie blue (lane 1), staining with Ponceau S (lane 2), Western blotting with mAb 36.4 (lane 3), immunoprecipitation with mAb 36.4, followed by immunodetection with pAb 982 (lane 4). IgA<sub>b</sub>, IgA polymer; IgA<sub>d</sub>, IgA dimer.

Binding Assay—The interaction between recombinant SC and IgA was determined by dot blot reassociation assay as described in Rindisbacher et al. (9). Murine IgA<sub>d</sub> and IgA<sub>b</sub> were obtained from hybridoma ZAC3 (8).

Measurement of IgA Transport by MDCK Cells in the Co-culture Assay—The procedure is based on the technique developed by Hirt et al. (10). MB-2/B7 hybridoma cells secreting IgA were cultivated as described (7). MDCK cells stably transfected with a pLKneo vector containing the wild-type or antigenized rabbit IgRcDNAs were induced to transfect (10). MB-2/B7 hybridoma cells secreting IgA were cultivated as described (7) and infected with recombinant adenovirus expressing immunoglobulin-like complementary determining regions (CDR) involved in IgA binding (12). We reasoned that since the loop tolerates important sequence variations, it might represent an ideal site for foreign epitope insertion.

In order to confirm that the loop indeed corresponded to the allele-specific epitope, we tested the ability of a synthetic peptide corresponding to amino acids 76–87 to compete with binding of a 161 allele-specific antiserum raised in 162 rabbits (17) against purified rabbit SC. The antiserum was incubated with 0, 140, or 280 ng of peptide, and the degree of competition was assessed by Western blotting and quantituated by scanning (Fig. 3B). Under non-reducing conditions, binding of the 161 allele-specific antibody to rabbit SC was only partially competed by the peptide. Competition was complete under reducing conditions, yet the affinity of the antibody for reduced SC is known to be lower (17). This suggests that the anti-161 antiserum recognizes a discontinuous surface epitope that includes all or part of the loop.

Selection of a Pathogen-specific Epitope—Several of the genes involved in invasion of cultured cells by Shigella flexneri have been identified and designated as *ipa* for “invasion plasmid antigen” (19, 20). The IpaB protein is exposed on the bacterial surface and results in antibody production in infected animals (21). For insertion into SC, we selected the immunodominant linear B cell epitope consisting of residues Lys-Asp-Arg-Thr-Leu-Ile-Glu-Gln-Lys of IpaB (22). This sequence changed the length of the original loop by only a single amino acid, is specifically recognized by mAb H10 (22), and remains accessible in the context of a β-galactosidase-IpaB fusion protein containing the entire IpaB polypeptide (Fig. 3C, lane 3).

Cloning of SC cDNA Sequences in a Vaccinia Virus Insertion Vector—Rabbit SC cDNA was engineered to contain the coding sequences for amino acids 1–571 fused to a C-terminal histidine hexamer tag (Fig. 4A). Following cloning into VV insertion plasmid pHGS1 (16), the fragment was introduced by homologous recombination into the TK locus of the viral genome. The presence of the rabbit SC cDNA insert in the viral genome was ascertained by PCR utilizing transfer vector-specific primers that generated unique amplification products of the expected sizes (Fig. 4B). Insertion of the IpaB epitope into the E-F loop of SC domain I was performed using PCR as described under “Experimental Procedures.”

Insertion of a Pathogen-specific Epitope into the First Domain of SC Reduces Its Secretion Rate by Infected CV-1 Cells—Heterologous production of SC was performed by infecting CV-1 cells with rRVs vvHGS1-SC68xHrs and vvHGS1-SC/ipaB68xHrs at a multiplicity of infection of 2 for 24 h. These conditions have been shown to be optimal for the expression of human SC using an identical approach (9). Secretion of wild-type or chimeric SC was assessed by immunoblotting. After 24 h of culture, wild-type SC could be detected in only a little as 5 µl of cell culture supernatant (Fig. 5A, lane 3), whereas no chimeric SC was detected under the same conditions (Fig. 5A, lane 1). 100-fold concentration of the medium was required to generate a chimeric SC signal of similar intensity to that obtained with cells infected with SC(wt) (Fig. 5A, lane 2), suggesting that processing and/or secretion was blocked within the surface of rabbit SC to insert a foreign epitope.

Selection of an Epitope Replacement Site on SC—In rabbit, three distinct SC alleles, t61, t62, and t63, have been identified (17, 18). The molecular model indicates that an 8-amino acid long sequence extending from leucine 79 to aspartic acid 86 (Fig. 3A) with the strongest allelic variation (>60% divergence when compared with other regions (2, 4)) is located within the loop connecting the E and F β strands. The loop is opposite to the stretches corresponding to the immunoglobulin-like complementary determining regions (CDR) involved in IgA binding (12). We reasoned that since the loop tolerates important sequence variations, it might represent an ideal site for foreign epitope insertion.
cells. To test this hypothesis, cell extracts were prepared in a final volume corresponding to that of the culture medium and analyzed for the presence of SC(IpaB)6xHis. 5 μl of extract generated a signal intensity on Western blot comparable with that of supernatant of vvHGS1-SC6xHis-infected cells. The chimeric intracellular SC migrated significantly faster than secreted wild-type SC on SDS-PAGE (Fig. 5A, lane 4), reflecting incomplete glycosylation and subsequent block in transport as a consequence of the alteration of the E-F loop in domain I. However, affinity purification of recombinant SC(IpaB) could be achieved on a Ni²⁺-NTA-agarose column (Fig. 5B) using the conditions given in Rindisbacher et al. (9).

**FIG. 2. Stereo ribbon representation of the first and second domain of the pIgR.** The C′-D loop and the E-F loop are shown in the front part of the model. The salt bridge linking arginine 63 and aspartic acid 86 is represented by a dotted line.

Traffic of the pIgR in MDCK cells Is Altered by the Insertion of the Foreign Epitope—We next examined whether the dramatic effect on secretion of replacing the E-F loop by an IpaB epitope observed in recombinant SC would hold true for the full pIgR. Receptor biosynthesis and its routing along the secretory pathway were analyzed in MDCK cells stably transfected with wild-type and chimeric receptors under the control of a glucocorticoid-inducible promoter (15). Transfected MDCK cells were grown to confluency on Transwell filters, and pIgR expression was induced by incubation with dexamethasone for 24 h. Intracellular accumulation of the receptor over the induction period was similar for the wild-type and chimeric proteins (Fig. 6A, lanes 1–4). In contrast, secretion of the chimeric SC into the apical medium was drastically reduced when compared with wild-type SC (Fig. 6A, lanes 5 and 6), in agreement with the results obtained for secretion of chimeric SC by CV-1 cells. When the stably transfected MDCK cells were examined by confocal laser scanning microscopy, wild-type pIgR exhibited strong perinuclear and cytoplasmic, as well as cell surface labeling (Fig. 6B). In contrast, chimeric pIgR was exclusively intracellular with a complete absence of surface staining (Fig. 6B). These data are consistent with an early block along the secretory pathway resulting in accumulation of endoglycosidase H-sensitive chimeric pIgR or SC in the rough endoplasmic reticulum or the cis-Golgi compartment (data not shown).

**FIG. 3.** Secretory IgA as a Vaccine Delivery System. Secretion of dimeric IgA by CV-1 cells infected with rVV (lanes 1–3) or by MDCK cells infected with wild-type SC (lanes 4–6). Secretion of SC(IpaB)6xHis (lanes 1 and 4) dropped to nearly undetectable levels, while secretion of SC(IpaB) was not altered (lanes 2 and 5). No secreted SC was detected in wild-type SC-infected CV-1 cells (lanes 3 and 6). The amounts of SC (SC1, SC2, SC3) and SC(IpaB)1, SC(IpaB)2, SC(IpaB)3, SC(IpaB)4 were measured in the apical medium by ELISA (Fig. 7A). The rate of IgA₃ transported by cells expressing the wild-type pIgR increased linearly to a plateau at 24 h, and ~150 ng IgA/filter were recovered from the apical medium. In contrast, no IgA or sIgA was detected in apical media from cells transfected with the chimeric receptor cDNA. The susceptibility of the chimeric receptor to cleavage was not increased, as reflected by the absence of chimeric SC in the basal medium. IgA₃ production by the hybridoma cells was not rate-limiting (Fig. 7B).

**FIG. 4.** The Pathogen-specific Epitope Inserted in SC Is Recognized by Its Cognate Monoclonal Antibody—To determine whether the amino acid substitution in the E-F loop altered the local structure in domain I, we tested whether the chimeric SC could still be recognized by the IpaB-specific mAb H10. Wild-type or antigenized SC in supernatants from rVV infected CV-1 cells were concentrated by immunoprecipitation using antibodies pAb 982, mAb 303, or mAb H10 and by trichloroacetic acid precipitation. Western blotting analysis showed that mAb H10 recognized antigenized SC in solution with an efficiency similar...
to pAb 982 and mAb 303. H10 failed to precipitate the wild-type protein, which was however immunoprecipitated by incubation with pAb 982 and mAb 303. Trichloroacetic acid-precipitated samples showed the same patterns when Western blots were probed with anti-SC antibodies, confirming that immunoprecipitation of recombinant SC was quantitative (Fig. 8A).

**Orally Administered IgA-SC(IpaB) Is Immunogenic**—In order to demonstrate that chimerized recombinant sIgA can serve as a mucosal delivery system, we immunized mice 4 times over a period of 2 months with 100 μg IgA(IpaB) together with cholera toxin as an adjuvant. Controls consisted of immunization with 100 μg IgA-SC(wt) together with cholera toxin as an adjuvant. Controls consisted of immunization with 100 μg IgA-SC(IpaB) containing lysate could be detected by incubation of the sera of the mice immunized with IgA-SC(IpaB) (Fig. 8B). This indicates that serum antibodies in mice immunized with IgA-SC(IpaB) can recognize the IpaB epitope in its native environment. Background levels were obtained with the sera of animals immunized with IgA-SC(IpaB) also mucosally. A strong specific response against the IpaB-containing lysate could be detected by incubation of the sera of the mice immunized with IgA-SC(IpaB).

**FIG. 3.** Panel A, sequence comparison of the rat and rabbit (alleles t61 and t62) pIgR between amino acids 76 and 89. Boxed residues form the E-F loop in domain I and exhibit the highest variability in the molecule. Mutations in the loop created by substitution with the IpaB peptide are shown for comparison. Panel B, titration of the anti-allelic t61 antiserum binding to native SC with increasing amounts of a synthetic peptide composed of amino acids 76–87 from the E-F loop. Western blots were probed with anti-t61 serum mixed with increasing amounts of peptide, and antibody binding was detected with secondary antibody coupled to horseradish peroxidase. The relative intensities of the signals arbitrarily set at 100 in the absence of competitor peptide were quantified by densitometry. Panel C shows that the mAb H10 recognizes a fusion protein consisting of E. coli galactosidase and S. flexneri invasin B.

**FIG. 4.** Panel A, schematic representation of SC constructs stably integrated in the VV genome. Abbreviations are: LP, leader peptide; 6xHis, C-terminal tag made of 6 histidyl residues; TKL and TKR, right and left arms of the vaccinia thymidine kinase gene; A, TK 5'-primer; and B, TK 3'-primer. Panel B, PCR amplification of three independent rVVs using primers A and B after the first (lanes 1–3), second (lanes 4–6), and third (lanes 8–10) round of plaque purification. Lane 7, amplification with pHGS1-SC6xHis plasmid used for generation of rVVs.

**FIG. 5.** Production of recombinant SC in CV-1 cells. Panel A, mobility of secreted and cell-associated wild-type and antigenized SC on Western blot. CV-1 cells were infected with VVs vvHGS1-SC6xHis and vvHGS1-SC(IpaB)6xHis for 24 h and then culture supernatants and cell lysates were resolved by SDS-PAGE. Blots of selected fractions were probed with the pAb 982. Lane 1, SC(IpaB) in unconcentrated supernatant; lane 2, SC(IpaB) in 100-fold concentrated culture supernatant; lane 3, wild-type SC in unconcentrated culture supernatant; lane 4, SC(IpaB) in the cell fraction. Panel B, Purification of SC(IpaB) by Ni²⁺-chelate affinity chromatography. Abbreviations are: L, load; FT, flow through; W, wash; E₄₀₀, elution with 40 mM imidazole; and E₈₀₀, elution with 80 mM imidazole.
bind to the free IpaB peptide, but not to the unrelated FLAG (Eastman Kodak) peptide (Fig. 8C). Saliva samples of two mice challenged with IgA-d-SC (IpaB) exhibited a reproducible, albeit weak, binding to Shigella lysates containing IpaB (Fig. 8D). Antibodies against SC were detected in all saliva samples of animals immunized with IgAd-SC (IpaB) or IgAd-SC (wt) (Fig. 8D). Data in Fig. 8B-D thus demonstrate that gastric delivery of antigenized sIgA can serve as a vaccine system capable of eliciting both a systemic and a mucosal antibody response.

**DISCUSSION**

The rationale for using SC as an epitope delivery system was based on the observation that oral administration of rabbit milk sIgA in mice triggered an immune response with antibodies directed against SC. As a basis to identify a site for epitope insertion at the surface of SC, we have predicted the three-dimensional structure of domain I. We selected a site mapping to a loop situated between the E and F \(^\beta\) strands (residues 79–86) opposite to the three CDR-like stretches in SC recently shown to be involved in IgA binding (12). This sequence also varies considerably among the different alleles of the rabbit pIgR. To test the hypothesis that epitopes inserted at this site would be immunogenic, this sequence was replaced by a S. flexneri IpaB epitope for which a monoclonal antibody was available (22).

Chimeric SC containing a Shigella invasin-specific epitope in its first domain retained the capacity to bind IgA\(_m\) but not IgA\(_m\) in both solution and on membrane support. Although insertion of the epitope did not alter the rate of synthesis of the chimeric SC or pIgR, the intracellular routing was compromised by the insertional mutation. This was reflected by a drastic reduction in the secretion rate of chimeric SC by rVV-infected CV-1 cells and by the absence of polymeric IgA transepithelial transport by MDCK cells expressing the receptor.

It has been established that the determinants required for non-covalent binding of polymeric IgA are restricted to the first most distal of the five immunoglobulin-like domains of pIgR and SC (24, 25). Amino acid substitutions in the three CDR-like loops in the first domain, and in the loop bridging strands E and F, drastically reduced IgA binding capacity of the pIgR (12). In contrast, the substitution in the E-F loop of amino acids...
79–86 by the IpaB sequence did not affect binding to IgA (this study). In addition, the mutation did not seem to induce significant long range structural changes in domain I since mAb 303, specific for a conformational epitope in the first domain (25), was able to bind as efficiently to chimeric and wild-type SC. This is in agreement with the results reported by Coyne et al. (12) in which mutations in the E-F loop of the pIgR did not affect binding of the same monoclonal antibody. Provided that the spatial organization of the E-F loop and C_0-D loop is stabilized by a salt bridge between the beginning of the D strand (Arg-63) and the end of the E-F loop (Asp-86), this suggests that Asp-86 plays a role in positioning the loop on the surface of the molecule rather than serving as a point of electrostatic interaction between SC and IgA.

Clearly, this salt bridge plays a crucial role in the folding of the first domain since its loss perturbs the intracellular trafficking of the chimeric SC or pIgR although IgA binding remains intact. For instance, interactions between domains I and II might be affected and hence interfere with SC and pIgR secretion. Alternatively, in the t61 allele-encoded protein, N-linked glycosylation takes place at a site 14 residues upstream of the E-F loop (Arg-63) and the C_0-D loop is stabilized by a salt bridge between the beginning of the D strand (Arg-63) and the end of the E-F loop (Asp-86), this suggests that the correct folding of the pIgR first domain is not required for binding to IgA, it is required for intracellular trafficking, most likely for exit from the endoplasmic reticulum compartment. Based on these considerations one should insert pathogen-specific epitopes elsewhere in SC to try to obtain a secretion-competent chimeric molecule. We are currently addressing this issue by mutating other loops and β strands in domains II and III. Indeed, the alternatively spliced low Mr pIgR (28) with deletion of domains 2 and 3 is functional (29) and binds non-covalently IgA. This suggests that these domains should be ideal alternative sites for peptide insertion or for replacement by larger foreign sequences containing both T and B epitopes.

Oral immunization is the most effective way to stimulate mucosal immunity in the intestine and at more distant sites including the genital tract and the lungs (30, 31). Usually, however, most soluble protein antigens are poorly immunogenic when given orally due to the systemic hyporesponsiveness, or oral tolerance, that is naturally generated (32). Mucosal adjuvants, such as the cholera toxin (CT) from Vibrio cholerae or the heat labile toxin from E. coli, are known to break tolerance (33, 34). Based on the observation that sIgA can bind to and travel across M cells in Peyer’s patches (7) and that CT favors switch differentiation to IgG1- and IgA-secretting cells (35, 36), we combined antigenized sIgA with CT to prevent tolerance. Under these conditions, we have been able to demonstrate that an epitope derived from a pathogenic bacte-
rrium inserted into recombinant sIgA survives in the proteolytic environment of the gut and elicits both a systemic and mucosal antibody response. Thus, SC associated with IgA can serve as a delivery vehicle for oral vaccination by preserving the immunogenicity of the inserted epitope. Interestingly, in the absence of adjuvant, keyhole limpet hemocyanin feeding, followed by subsequent subcutaneous immunization, induced systemic T cell tolerance but induced B cell priming at both systemic and mucosal sites (37). Whether a similar mechanism might take place with antigenized sIgA remains to be determined. Since topically administered sIgA can be used for the prevention of viral and bacterial infection (38–41), it now becomes possible to combine passive immunization with active mucosal vaccination.

Acknowledgments—We gratefully acknowledge the expertise of Irène Corthésy-Theulaz and Nadine Porta for the mouse immunization experiments. We thank Monique Reinhardt for excellent technical assistance and Sally Hopkins and Pascal Crottet for critical reading of the manuscript.

REFERENCES

1. Neutra, M. R., Michetti, P., and Kraehenbuhl, J.-P. (1994) in "Physiology of the Gastrointestinal Tract" (Johnson, L. R., ed) p. 685, Raven Press, Ltd., New York
2. Mostov, K. E., Friedlander, M., and Blobel, G. (1984) Nature 308, 37–43
3. Banting, G., Brake, B., Braghetta, P., Luzio, J. P., and Stanley, K. K. (1989) FEBS Lett. 254, 177–183
4. Schaerer, E., Verrey, F., Racine, L., Talichet, C., Rheinhardt, M., and Kraehenbuhl, J.-P. (1990) J. Cell Biol. 110, 987–998
5. Kraeci, P., Gresschik, K. H., Geurts van Kessel, A. H. M., Olaisen, B., and Brandtzaeg, P. (1991) Hum. Genet. 87, 642–648
6. Roy, M. J., and Varvayanis, M. (1987) Cell Tissue Res. 248, 645–651
7. Weltzin, R., Lüthi, R., Bowie, J. U., and Eisenberg, D. (1992) J. Cell Biol. 106, 1673–1685
8. Weltzin, R., Lucia-Jandris, P., Fields, B. N., Kraehenbuhl, J.-P., and Neutra, M. R. (1993) J. Immunol. 142, 1087–1090
9. Hirt, R., Hughes, G. J., Prutiger, S., Michetti, P., Perregaux, C., Poulain-Godefroy, O., Jeanguenat, N., Neutra, M. R., and Kraehenbuhl, J.-P. (1993) Cell 74, 245–255
10. Harlow, F., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
11. Neutra, M. R., Michetti, P., and Kraehenbuhl, J.-P. (1994) in "Cellular and Molecular Approaches to the Gastrointestinal Tract" (Johnson, L. R., ed) p. 685, Raven Press, Ltd., New York
12. Coyne, R. S., Siebrecht, M., Peitsch, M. C., and Casanova, J. E. (1994) J. Biol. Chem. 269, 31620–31625
13. Luthi, R., Bowie, J. U., and Eisenberg, D. (1992) Nature 356, 83–85
14. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
15. Hirt, R., Hughes, Poulain-Godefroy, O., Billotte, J., Kraehenbuhl, J.-P., and Fasel, N. (1992) Gene (Amst.) 111, 199–206
16. Berthelet, C., Drillien, R., and Wittek, R. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2096–2099
17. Knight, K. L., Rosenzweig, M., Lichter, E. A., and Hanly, W. C. (1974) J. Immunol. 112, 877–882
18. Hanly, C. W., Cook, L., Kingzette, M., Cox, W., Frutiger, S., Hughes, G. J., and Jaton, J.-C. (1987) J. Immunol. 139, 1597–1601
19. Maurelli, A. T., Baudry, B., d’Hauterive, H., Hale, T. L., and Sansonetti, P. J. (1985) Infect. Immun. 49, 164–171
20. Baudry B., Maurelli A. T., Clerc P., Sadoff J. C., and Sansonetti, P. J. (1987) J. Gen. Microbiol. 133, 3493–3413
21. Philaplon, A., Arndel, J., Nato, F., Rouyre, S., Mazie, J. C., and Sansonetti, P. J. (1992) Infect. Immun. 60, 1919–1926
22. Barza, S., Nato, F., Rouyre, S., Mazie, J. C., Sansonetti, P. J., and Philaplon, A. (1993) Infect. Immun. 61, 3825–3831
23. Bakos, M.-A., Widen, S. G., and Goldblum, R. M. (1994) Mol. Immunol. 31, 165–168
24. Frutiger, S., Hughes, G. J., Hanly, W. C., Kingzette, M., and Jaton, J.-C. (1996) J. Biol. Chem. 261, 16673–16681
25. Kuhn, L. C., Kocher, H. P., Hanly, W. C., Cook, L., Jaton, J.-C., and Kraehenbuhl, J.-P. (1983) J. Biol. Chem. 258, 6653–6659
26. Frutiger, S., Hughes, G. J., Hanly, W. C., and Jaton, J.-C. (1988) J. Biol. Chem. 263, 8120–8125
27. Hammond, C., and Helenius, A. (1994) Cell Biol. 126, 41–52
28. Deitzer, D. L., and Mostov, K. E. (1990) Mol. Cell. Biol. 6, 2712–2715
29. Solari, R., and Kraehenbuhl, J.-P. (1987) in "The Mammary Gland" (Weisbrod, G., and Levine, M., eds) p. 217, Marcel Dekker, Inc., New York
30. McGehee, J. R., Mestecky, J., Dertzbaugh, M. T., Eldridge, J. H., Hirasewa, M., and Kiyono, H. (1992) Vaccine 10, 75–88
31. Weiner, H. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10762–10765
32. Hansen, L. A., Teleno, E., Wiedermann, U., Dahlman, A., Saulmann, R., Ablstedt, S., Friman, V., Holmgren, J., Czerkinsky, C., and Dahlgren, U. (1993) Pediatr. Allergy Immunol. 4, 16–20
33. Sun, J. B., Holmgren J., and Czerkinsky, C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10795–10799
34. Lycke, N., Severinsson, E., and Streicher, W. (1990) J. Immunol. 145, 3516–3524
35. Pierre, P., Denis, O., Bains, R., Iminu, N., Meikle, B., and Vearnson, J.-P. (1992) Eur. J. Immunol. 22, 3179–3182
36. Hruby, S., Mestecky, J., Maloveaneau, Z., Holland, S., and Elson, C. O. (1994) J. Immunol. 152, 4663–4670
37. McGehee, J. R., Mestecky, J., Dertzbaugh, M. T., Eldridge, J. H., Hirasewa, M., and Kiyono, H. (1992) Vaccine 10, 75–88
38. Weiner, H. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10762–10765
39. Michetti, P., Mahan, M. J., Slautch, J. M., Mekalanos, J. J., and Neutra, M. R. (1992) Infect. Immun. 60, 1786–1792
40. Hammarström, V., Smith, C. I., and Hammarström, L. (1993) Lancet 341, 1036
41. Weltzin, R., Hsu, S. A., Mittler, E. S., Georgakopoulos, K. M., and Monath, T. P. (1994) Antimicrob. Agents Chemother. 38, 2785–2791
42. Solari, R., Kuhn, L. C., and Kraehenbuhl, J.-P. (1985) J. Biol. Chem. 260, 1141–1145