# Characterization of the Recombinant Extracellular Domains of Human Interleukin-20 Receptors and Their Complexes with Interleukin-19 and Interleukin-20[†]

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ABSTRACT: The soluble extracellular domains of human interleukin-20 (IL-20) receptors I and II (sIL-20R1 and sIL20R2), along with their ligands IL-19 and IL-20, were expressed in *Drosophila* S2 cells and purified to homogeneity. Formation of the receptor/receptor and ligand/receptor complexes was studied by size exclusion chromatography. Both ligands and soluble receptors were found to be monomeric in solution; homo- or heterodimers are not formed even at elevated concentrations. Under native conditions, both IL-19 and IL-20 form stable ternary 1:1:1 complexes with the sIL-20R1 and sIL20R2 receptors, as well as high-affinity binary complexes with sIL-20R2. Unexpectedly, sIL-20R1 does not bind on its own to either IL-19 or IL-20. Thus, one of the possible consecutive mechanisms of formation of the signaling ternary complex may involve two steps: first, the ligand binds to receptor II, creating a high-affinity binding site for the receptor I, and only then does receptor I complete the complex.

IL-19 (*1*) and IL-20 (*2*) are cytokines considered to be related to IL-10 (*3*) on the basis of their sequence homology. Other known members of the IL-10 family are IL-22 (*4*, *5*), IL-24 (*6*), and IL-26 (*7*). The family also includes several viral homologues of IL-10 derived from large DNA viruses, in particular, herpesviruses and poxviruses, including Epstein−Barr virus (*8*, *9*), equine herpesvirus type 2 (*10*), Orf parapoxvirus (*11*, *12*), human and simian cytomegaloviruses (*13*, *14*), and Yaba-like disease virus (*15*). Amino acid sequences of the recently discovered interferons IFN-$\lambda$1, IFN-$\lambda$2, and IFN-$\lambda$3 (also named IL-29, IL-28A, and IL-28B) (*16*, *17*) also show limited homology to the members of the IL-10 family.

Formation of complexes with specific membrane-spanning receptors that belong to the class II cytokine receptor family (*18*) is an initial step in the signaling processes that involve these cytokines. To create signaling complexes, two distinct receptor subunits (type I and type II) are required. These receptor types differ mainly in the sizes of their intracellular domains and in signaling capabilities. For receptors using members of both the IL-10 and INF-$\gamma$ families as ligands, the customary assignment of the receptors as type I (*19*) was based on the presence of longer intracellular domains that were able to recruit STATs. Accordingly, a receptor with a shorter intracellular domain that did not interact with STATs was named type II (*20*). It has also been shown that, despite their different biological activities, some of these cytokines share their receptors with each other and sometimes utilize the same ones. For example, the second receptor of IL-10, IL-10R2, is involved in the formation of signaling complexes of not only IL-10 but also IL-22 and INF-$\lambda$s, whereas the first receptor of IL-22, IL-22R1, also participates in a complex formation induced by IL-20 and IL-24 (*21*−*24*). Both IL-20 and IL-24 form complexes with the receptor pair IL-22R1/IL-20R2 (*25*). In addition, IL-19, IL-20, and IL-24 also signal through formation of the complexes with IL-20R1/IL-20R2 (*2*, *23*, *25*).

IL-19 was detected in immune cells such as LPS-stimulated and resting peripheral blood mononuclear cells. The fact that EBV-transformed B cell cDNA was used as a source for the initial cloning of IL-19 demonstrates that B cells can also synthesize this cytokine (*1*). This observation was confirmed in experiments showing that IL-19 has low expression levels in rested and stimulated B cells (*26*). It is likely that some novel functions of IL-19 have yet to be determined, but it is already clear that this cytokine plays a significant role in the immune system.

IL-20 plays an important role in skin biology. Overexpression of IL-20 in transgenic mice resulted in neonatal lethality with skin abnormalities, similar to those found in psoriatic skin (*2*). By contrast, it was reported that IL-19-

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overexpressing transgenic mice had no overt skin phenotype (*27*).

A molecule of IL-19 is a monomer consisting primarily of seven amphipathic helices arranged in a compact seven-helix bundle (*28*). Comparison of the amino acid sequences of IL-19 and IL-20 (44% identity, 52.5% homology) suggests that their three-dimensional structures must be very similar. To address the question of ligand/receptor complex formation and how structurally similar cytokines induce quite different biological events by using the same receptors, we expressed and purified extracellular domains of IL-20R1 and IL-20R2 receptors and studied their interactions with both the IL-19 and IL-20.

## EXPERIMENTAL PROCEDURES

*Expression and Purification of IL-19, IL-20, sIL-20R1, and sIL-20R2.* Expression and purification of both the ligands and the soluble receptors have essentially followed previously described protocols (*28*). Human cDNA encoding an appropriate mature protein was combined with the sequence for the *Drosophila* BiP leader peptide. An expression tag consisting of six histidine residues was placed at the N-terminus of a ligand or at the C-terminus of a receptor, while an artificial opal (tga) stop codon was introduced at the 3′ end of each expressed sequence. Engineered sequences were cloned into the insect cell expression vectors pAc5.1/V5-HisA or pMT/BiP/V5-His (Invitrogen) and transfected into S2 cells using the cationic lipid Maxfect (Molecular Research Laboratories, Inc., Herndon, VA). pCo-Hygro or blasticidin S plasmid (Invitrogen) was also transfected into the cells to allow drug selection. Media containing an appropriate antibiotic were changed every 72 h until the amount of antibiotic-resistant cells in tissue culture plates and flasks reached approximately $1 \times 10^8$. Subsequently, cells were propagated without antibiotics as spinner cultures at densities between $1 \times 10^6$ and $5 \times 10^7$/mL. Proteins were purified by metal affinity chromatography (Ni or Cu), followed by size exclusion chromatography, yielding 1−3 mg of pure protein/L of conditioned media.

*Size Exclusion Chromatography.* Size exclusion chromatography was performed on a Superdex 75 HiLoad (16/60) column (Pharmacia). The system was calibrated with the Amersham Pharmacia Biotech low molecular weight calibration kit, which includes bovine serum albumin (67 kDa), hen egg ovalbumin (43 kDa), bovine pancreatic chymotrypsinogen A (25 kDa), and bovine pancreatic ribonuclease A (13.7 kDa). The void volume of the column was determined with Blue Dextran 2000. Prior to each experiment, the column was equilibrated with 2 bed volumes of the elution buffer (50 mM HEPES, pH 7.5, 200 mM NaCl, 1 mM $NaN_3$). All separations were performed at the flow rate of 0.33 mL/min. One bed volume was collected as 1 mL fractions after injection of the sample. The peaks were analyzed on SDS−PAGE and processed with silver stain.

*Preparation and Purification of the Receptor/Ligand and the Receptor/Receptor Complexes.* Both binary and ternary complexes were prepared by mixing appropriate components with an excess of the heavier one, followed by incubation at room temperature for 2 h. The resulting material was centrifuged for 15 min at 13000 rpm before injection on the gel filtration column.

## RESULTS AND DISCUSSION

*Choice of Method and Columns.* To study the binding of IL-19 and IL-20 with their receptors, we used size exclusion chromatography even though it would not give us quantitative assessment of the binding properties of the molecules involved. However, gel filtration can be exploited for qualitative estimation of aggregation states of individual compounds as well as comparable evaluation (i.e., stronger or weaker) of the affinities between the components of studied complexes. Therefore, if we assume that molecules with higher affinities most likely would interact first, it is possible to predict the sequence of binding events during complex formation on the basis of the data of gel filtration.

Preparative size exclusion columns were given preference as they are longer than analytical columns and thus can better separate components with close molecular masses. Our first experiments on ternary complexes of both IL-19 and IL-20 have been performed with a Superdex 200 (16/60) column. Since no eluates with $M_r > \sim 90$ kDa were found, to increase separation of the lower molecular mass components, we continued with the Superdex 75 (16/60) column having lower molecular mass range.

*State of Aggregation and Glycosylation of Individual Molecules.* Since the last step in all of the protein purification procedures involved gel filtration, there was no need to perform additional chromatography to determine the aggregation states of the samples. IL-19 and IL-20 were each eluted from the gel filtration column as a single peak at elution volumes of 75−77 mL, corresponding to molecular masses of 19−22 kDa. The respective elution volumes of sIL-20R1 and sIL-20R2 were 67−68 and 71−72 mL, corresponding to molecular masses of 35 and 26 kDa. Since the molecular masses of single polypeptide chains of IL-19, IL-20, sIL-20R1, and sIL-20R2, estimated by SDS−PAGE under denaturing conditions, correspond well to the data obtained by gel filtration, we may conclude that both the ligands and the receptors are monomers in solution.

The number of putative glycosylation sites varies widely among these proteins. Two such sites are present in the sequence of IL-19, none in IL-20, two in sIL-20R2, and six in sIL-20R1. The crystal structure of IL-19 (*28*) showed that only the Asn-38 site is glycosylated, whereas Asn-117 is not, which is in good agreement with the presence of two bands corresponding to IL-19 in SDS−PAGE (Figure 1a). Since sIL-20R2 also yields only two bands in SDS−PAGE with molecular masses higher than the theoretical molecular mass of a nonglycosylated protein (25 and 26 kDa vs 23.6 kDa), we may assume that sIL-20R2 protein expressed in S2 cells has either one or both glycosylation sites occupied by carbohydrates. The calculated molecular mass of the sIL-20R1 is 26.8 kDa; however, this soluble receptor produced multiple bands between 30 and 35 kDa on SDS−PAGE. The difference between the theoretical molecular mass and the mass estimated from the gel indicates that the majority of the glycosylation sites are occupied.

*Interactions of IL-19 and IL-20 with sIL-20R1.* Binary complexes of IL-19 and IL-20 with sIL-20R1 were prepared with an excess of the receptor, usually at the molar ratio of 1:2. This was done to ensure that if a complex were to be formed, then the heavier molecule of sIL-20R1 would elute last. If, however, the molecules would not bind each other,

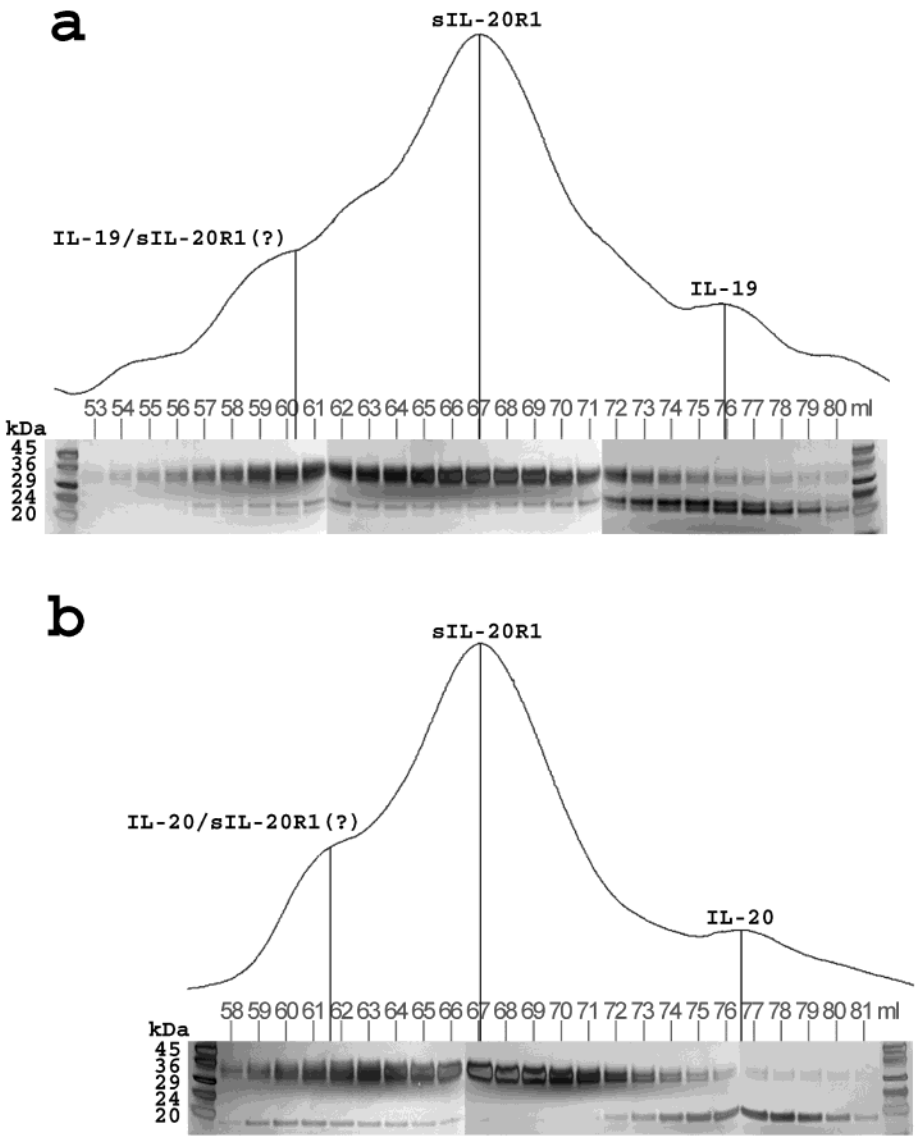


FIGURE 1: Gel filtration chromatography analysis of a mixture of either IL-19 or IL-20 with sIL-20R1. (a) IL-19 and sIL-20R1. The first peak (60 mL) is an overlap of the weak IL-19/sIL-20R1 complex and sIL-20R1 peak. SDS−PAGE of corresponding fractions shows the presence of both sIL-20R1 (multiple bands between 30 and 35 kDa) and IL-19 (20 and 22 kDa). Peak 2 (67 mL) corresponds to sIL-20R1. Peak 3 (76 mL) corresponds to the free IL-19. (b) IL-20 and sIL-20R1. The first peak at 61.5 mL is an overlap of a weak IL-20/sIL-20R1 complex and sIL-20R1. The second and the third peaks are free sIL-20R1 and IL-20, respectively.

then the lighter molecule, IL-19 or IL-20, would elute last. The IL-19/sIL-20R1 sample produced three overlapped peaks with their centers located at volumes of 60, 67, and 76 mL (Figure 1a). These elution volumes correspond to the molecular masses of 60, 35, and 19 kDa. SDS−PAGE shows that the first peak contains the receptor and a small amount of ligand, whereas the second and the third peaks correspond to the free receptor and the free ligand, respectively.

The IL-20/sIL-20R1 sample eluted off the column as three peaks corresponding to the absorption maxima at volumes of 61.5, 67, and 76.5 mL (Figure 1b). As was the case of the IL-19/sIL-20R1 complex, the first peak contains both a small fraction of IL-20 and a small fraction of sIL-20R1, whereas the second and the third peaks are the free receptor and the free ligand, respectively.

Since in both experiments the majority of the material eluted off the gel filtration column as separate soluble receptor and ligand peaks, we conclude that neither IL-19 nor IL-20 was capable of forming strong binary complexes with sIL-20R1, although the presence of the first minor peak containing a small amount of ligand indicates that some weak interaction of IL-19 or IL-20 with sIL-20R1 is still possible. To preclude the possibility that formation of the complex was too slow to be observed, we repeated the experiment after incubating the mixture of the IL-19 or IL-20 with sIL-20R1 at room temperature for 12 h prior to its injection onto the gel filtration column. Both chromatography and SDS−PAGE gave exactly the same results as in the 2 h experiment. We thus conclude that any trace amounts of the binary complexes (IL-19/sIL-20R1, IL-20/sIL-20R1) are very likely artifacts of the particular experimental conditions, such as high protein concentration, but are not biologically relevant. Our results agree with the data showing that sIL-20R1 cannot inhibit the activity of IL-19 or IL-20 in competition luciferase assays (*27*).

*Interactions of IL-19 and IL-20 with sIL-20R2.* Analogous experiments were performed in order to determine whether sIL-20R2 could form stable complexes with IL-19 or IL-

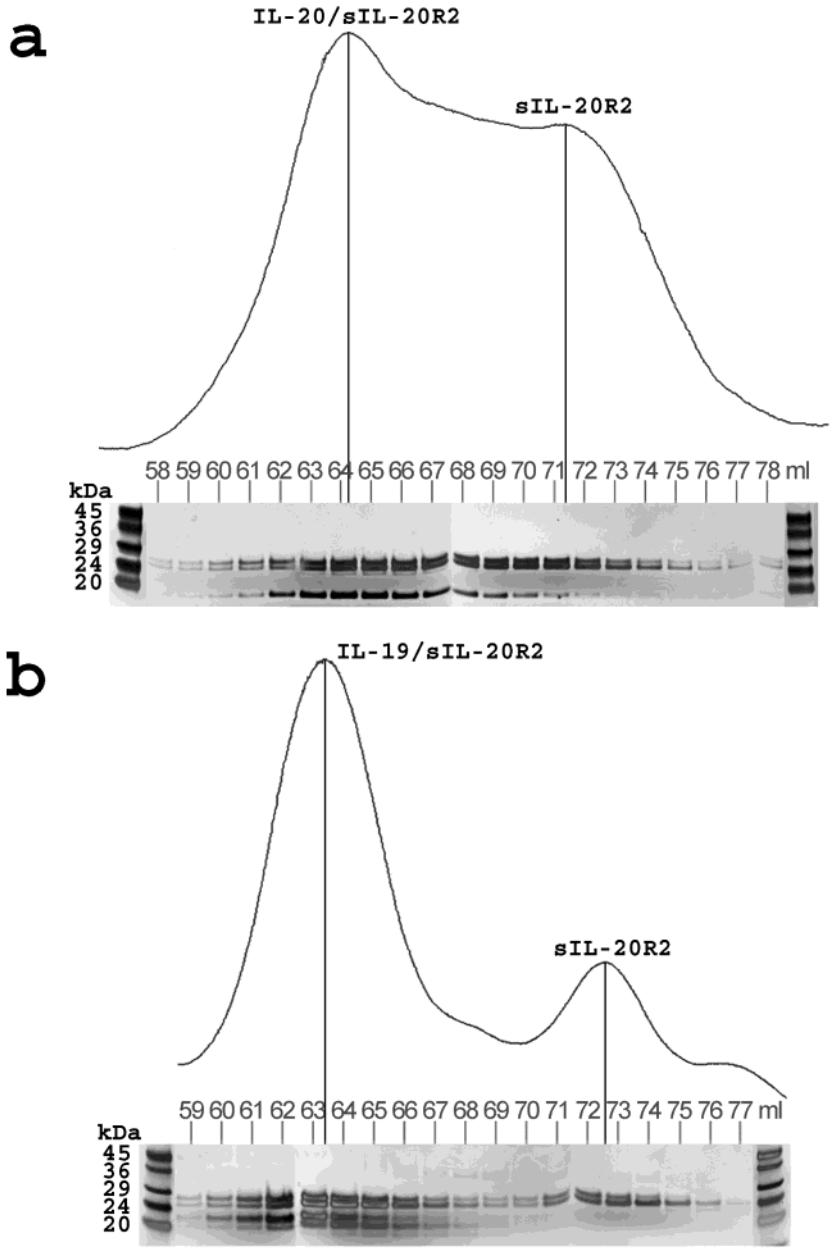


FIGURE 2: Gel filtration chromatography analysis of mixtures of either IL-19 or IL-20 with sIL-20R2. (a) IL-20/sIL-20R2 sample. (b) IL-19/sIL-20R2 sample. Both samples were eluted as two peaks. The first peak (64 mL for IL-20/sIL-20R2 and 63.5 mL for IL-19/sIL-20R2) corresponds to the binary ligand/second receptor complex. SDS−PAGE analysis of corresponding fractions shows the presence of both ligands and receptor (24 and 26 kDa species for sIL-20R2, 19 kDa species for IL-20, and 20 and 22 kDa species for IL-19). The second peak in both experiments (71.5 and 72.5 mL) corresponds to an excess of free sIL-20R2.

20. Both cytokines were mixed with an excess of sIL-20R2, incubated at room temperature for 2 h, and passed through the gel filtration column.

The sample of the IL-20/sIL-20R2 mixture eluted as two overlapped peaks with the centers at volumes of 64 and 71.5 mL (Figure 2a) while IL-19/sIL-20R2 sample eluted as two separate peaks at 63.5 and 72.5 mL (Figure 2b). The elution pattern of the IL-19/sIL-20R2 complex was quite similar to that of the sample of IL-20/sIL-20R2 except that the excess of free soluble receptor was smaller and the peaks corresponding to the complex and free receptor were completely separated. Individual fractions of the peaks from both experiments were analyzed by SDS−PAGE and showed the presence of both the ligand and the receptor in the first peak, while only free sIL-20R2 was seen in the second peak (Figure 2). The major part of the binary complexes eluted from the column as fractions corresponding to molecular mass 45−50 kDa. In addition, SDS−PAGE of the binary complex fractions showed similar intensity of the bands representing the ligand and the receptor, suggesting an equimolar ratio of the individual components of the sample (Figure 2). Taken together, this indicates that the binary complexes between sIL-20R2 and either IL-19 or IL-20 consist of a single cytokine molecule bound to a single molecule of the soluble receptor. Since the last component to be eluted from the column was sIL-20R2, we conclude that all of the cytokine ligand must remain bound. Thus, contrary to what we observed for sIL-20R1 that did not appear to stay bound to either IL-19 or IL-20, sIL-20R2 forms complexes with both ligands although broadening of the IL-20/sIL-20R2 peak (Figure 2a) may indicate that the IL-19/sIL-20R2 complex is more stable.

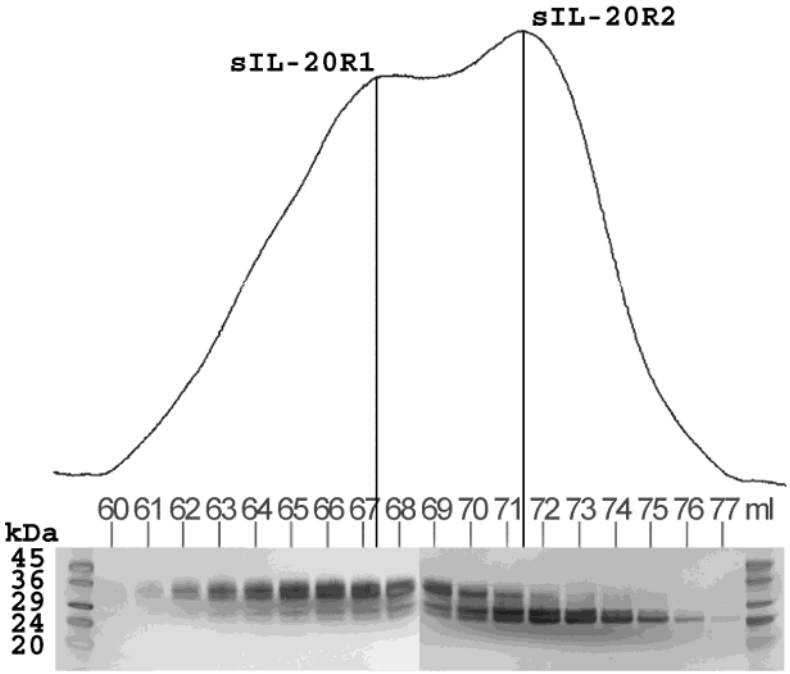


FIGURE 3: Gel filtration chromatography analysis of the mixture of free receptors. The first peak (67.5 mL) corresponds to free sIL-20R1, whereas the second peak (71.5 mL) corresponds to free sIL-20R2.

Competition luciferase assays performed on BHK570 cells with stably transfected receptors showed that a >1000-fold excess of sIL-20R2 is capable of blocking IL-19 activity, suggesting that sIL-20R2 competes for IL-19 with the full-length receptor on the cell surface (*27*). However, sIL-20R2 had no effect on the activity of IL-20 at any concentration that was tried in the same cells transfected with the same receptors. Nevertheless, our data show that sIL-20R2 does form a binary complex with IL-20 and that its properties are similar to the IL-19/sIL-20R2 complex.

The chromatograms of both the IL-19/sIL-20R2 and the IL-20/sIL-20R2 complexes also contain minor peaks eluting at volumes of 53−54 mL, only slightly higher than the background (data not shown). These peaks, much weaker than the major peaks corresponding to the 1:1 complexes discussed above, indicate the molecular mass to be in the range of 90−95 kDa. SDS−PAGE showed that the fractions collected between 51 and 57 mL contain approximately equimolar amounts of the ligand and the receptor, which, together with the estimated molecular mass of the components, suggests that the peaks could represent the complex consisting of two molecules of the ligand and two receptors. However, since the UV absorption signal corresponding to these peaks is very weak, it is likely that the larger complex could be an artifact due to the particular experimental conditions and resulting from nonspecific interactions.

*sIL-20R1 and sIL-20R2 Do Not Interact with Each Other.* When an equimolar mixture of sIL-20R1 and sIL-20R2 was incubated at room temperature for 8 h and subsequently passed through a gel filtration column, it eluted as two overlapping peaks centered at volumes of 67.5 and 71.5 mL, corresponding to the molecular masses of 35 and 26 kDa (Figure 3). The difference between the molecular masses of the two components does not allow their complete separation by gel filtration; however, when fractions 60−77 were analyzed on SDS−PAGE (Figure 3), it become clear that the receptors do not form any complex without the ligand present.

*Ternary Complexes IL-19/sIL-20R1/sIL-20R2 and IL-20/sIL-20R1/sIL-20R2.* It has been commonly accepted that cytokines bind to their receptors in a consecutive fashion, first forming a 1:1 complex with the high-affinity receptor and only then binding the second receptor, ultimately initiating signaling events. Since neither IL-19 nor IL-20 binds sIL-20R1 on its own, but both form stoichiometric complexes with sIL-20R2, we decided to investigate whether the binary complex represents an intermediate in the formation of the relevant ternary complex.

An excess of sIL-20R1 was added to the binary complexes of either IL-19/sIL-20R2 or IL-20/sIL-20R2, followed by incubation at room temperature for 2 h. The samples were then loaded on a gel filtration column and eluted as two distinct peaks at volumes of 56.5 and 68 mL, corresponding to the molecular masses of 75−85 and 35 kDa (Figure 4). As analyzed by SDS−PAGE, the first peak contained equal amounts of the two receptors and the ligand, while only sIL-20R1 could be detected in the second peak. We may therefore conclude that ternary complexes can be formed via binding of the first receptor to a binary complex of either IL-19 or IL-20 with sIL-20R2. On the basis of the apparent molecular mass of 75−85 kDa, the complex consists of one ligand, one molecule of sIL-20R1, and one molecule of sIL-20R2. It should be noted that there was no indication of any 2:2 binary complexes mentioned above, confirming that they were most likely artifacts.

Mixing of either IL-19 or IL-20 with both soluble receptors resulted in rapid formation of a ternary complex (30 min at room temperature). As in the previous experiment, the complex eluted at a column volume of 56−57 mL, corresponding to the molecular mass of 75−85 kDa. This indicates the presence of the ternary complexes consisting of IL-19/sIL-20R1/sIL-20R2 or IL-20/sIL-20R1/sIL-20R2 in 1:1:1

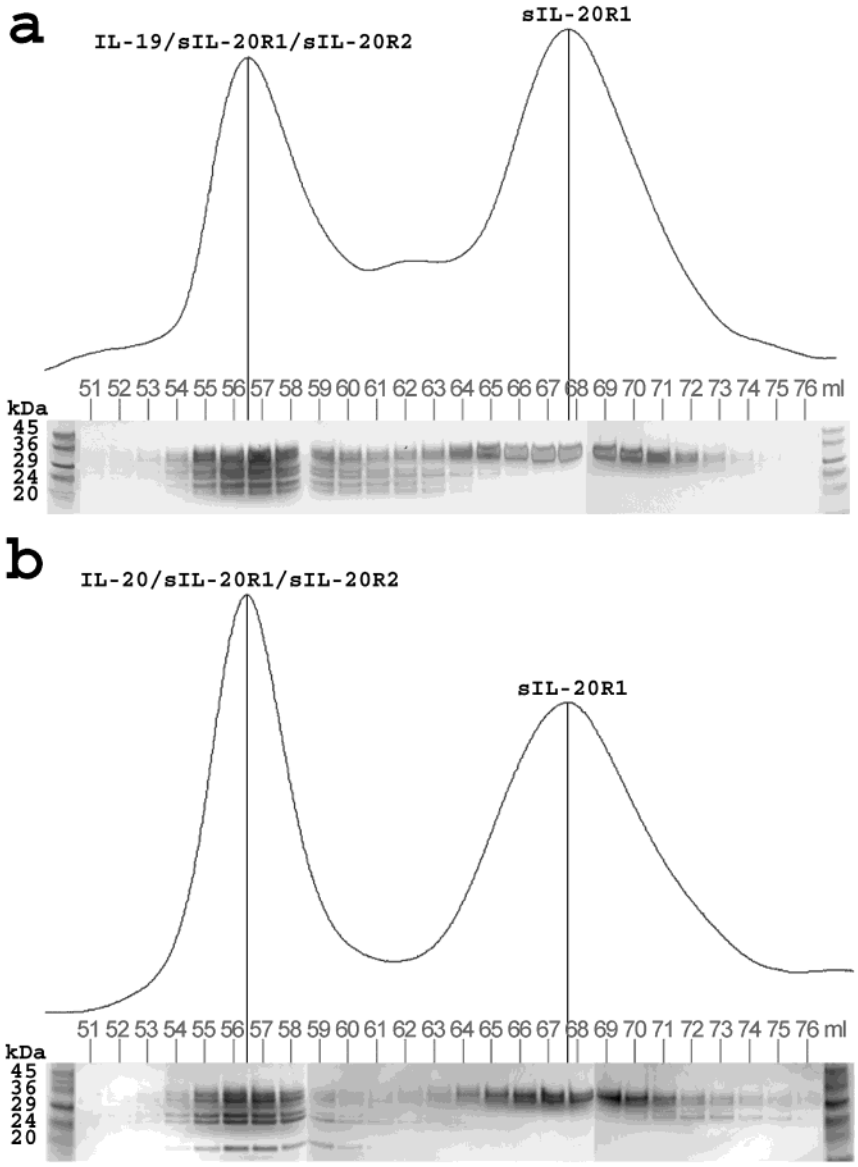


FIGURE 4: Gel filtration chromatography analysis of a ternary complex formed by combining binary IL-19/sIL-20R2 or IL-20/sIL-20R2 complexes with sIL-20R1. (a) IL-19/sIL-20R2 plus sIL-20R1. The first peak corresponds to the ternary IL-19/sIL-20R1/sIL-20R2 complex (56.5 mL), and the second peak corresponds to free sIL-20R1 (68 mL). SDS−PAGE analysis shows the presence of IL-19 (20 and 22 kDa bands), sIL-20R1 (multiple bands between 30 and 35 kDa), and sIL-20R2 (24 and 26 kDa bands) in the first peak and an excess of free sIL-20R1 in the second peak. (b) IL-20/sIL-20R2 plus sIL-20R1. The first peak corresponds to the ternary IL-20/sIL-20R1/sIL-20R2 complex (56.5 mL) and the second to free sIL-20R1 (68 mL). SDS−PAGE analysis shows the presence of IL-20 (19 kDa), sIL-20R1 (multiple bands between 30 and 35 kDa), and sIL-20R2 (24 and 26 kDa bands) in the first peak and an excess of free sIL-20R1 in the second peak.

stoichiometry (Figure 5). The IL-19/sIL-20R1/sIL-20R2 complex was prepared with the excess of the ligand; the sample yielded the second peak with two absorption maxima at 75 and 77 mL (Figure 5a) corresponding mostly to the heavier, glycosylated species of IL-19 and the mixture of glycosylated and nonglycosylated molecules. An excess of the free soluble receptors in the IL-20/sIL-20R1/sIL-20R2 sample gave two usual peaks at 67.5 and 71 mL corresponding to the first and the second receptor (Figure 5b), respectively.

These experiments have clearly shown that neither IL-19 nor IL-20 interacts with sIL-20R1 in the absence of sIL-20R2. The two receptors also do not interact with each other without a ligand present, whereas both IL-19 and IL-20 are capable of forming binary complexes with sIL-20R2. Addition of sIL-20R1 to either the IL-19/sIL-20R2 or IL-20/sIL-20R2 binary complex results in the formation of ternary complexes which are identical to those formed by IL-19 or IL-20 when the ligands and two receptors are mixed together, even for a short time. It is very likely that the normal sequence of events during ternary complex formation is such that IL-19 or IL-20 binds to IL-20R2 first, creating a new interaction site for IL-20R1. Binding of the latter completes creation of the final complex and may initiate signaling events. On the basis of its molecular mass, the ternary complex is likely to consist of one molecule of the ligand and one molecule of each receptor.

Our results are in good agreement with previous experiments aimed at the determination of which soluble receptors were capable of blocking ligand activity (*27*). In this set of experiments, BHK570 cells were stably transfected with IL-20R1/IL-20R2 and a reporter construct consisting of the firefly luciferase gene driven by promoter/enhancer sequences comprised of tandem STAT-binding elements. Cells

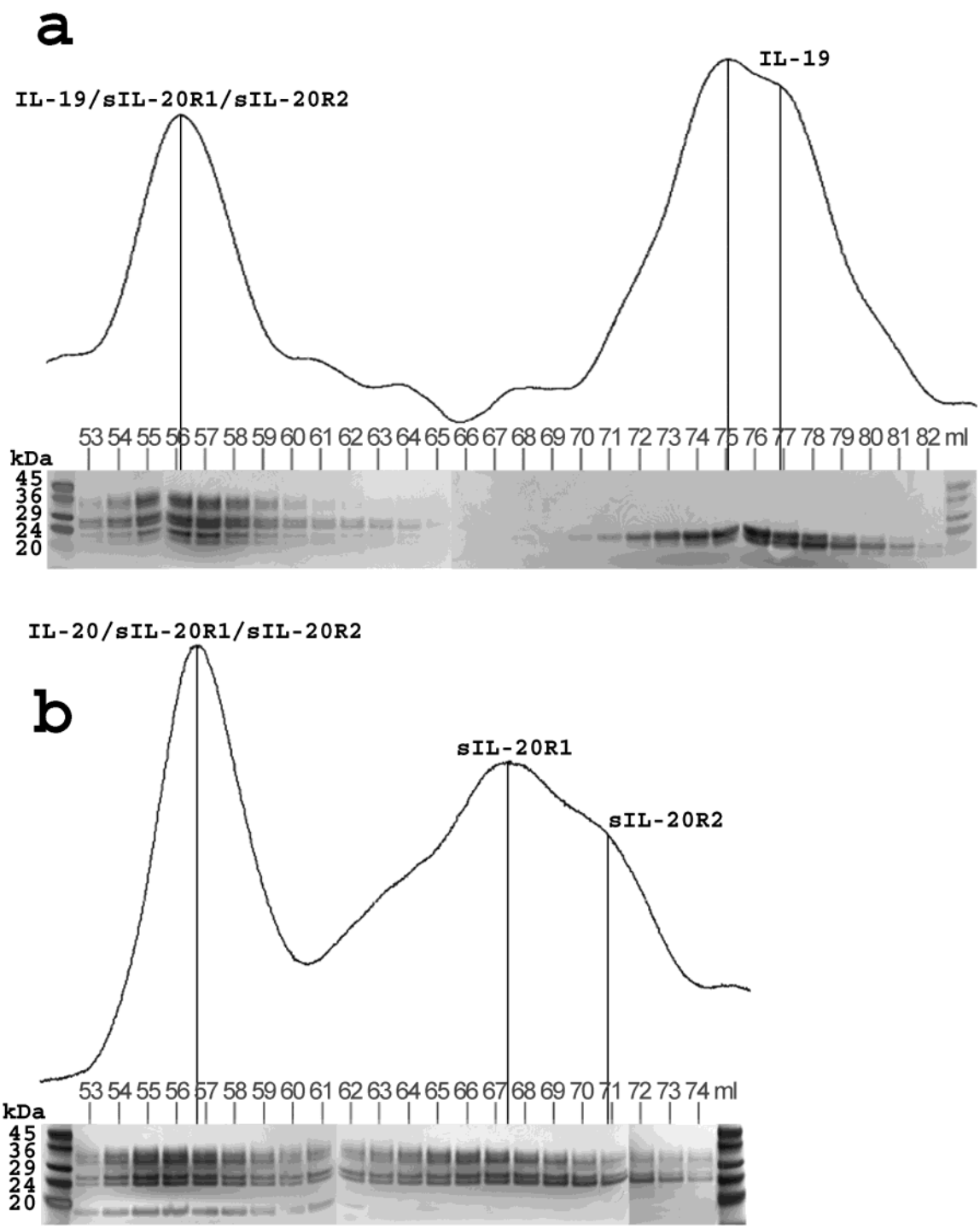


FIGURE 5: Gel filtration chromatography analysis of a ternary complex formed by combining of free IL-19 or IL-20 with a mixture of sIL-20R1 and sIL-20R2. (a) IL-19/sIL-20R1/sIL-20R2 sample. The first peak corresponds to the ternary IL-19/sIL-20R1/sIL-20R2 complex (56 mL), and the second peak is free IL-19 (75−77 mL). SDS−PAGE analysis shows the presence of IL-19 (20 and 22 kDa bands), sIL-20R1 (multiple bands between 30 and 35 kDa), and sIL-20R2 (24 and 26 kDa bands) in the first peak and free IL-19 in the second peak. (b) IL-20/sIL-20R1/sIL-20R2 sample. The first peak corresponds to the ternary IL-20/sIL-20R1/sIL-20R2 complex (56.5 mL), the second peak corresponds to free sIL-20R1 (67.5 mL), and the third peak corresponds to free sIL-20R2 (71 mL). SDS−PAGE analysis shows the presence of IL-20 (19 kDa), sIL-20R1 (multiple bands between 30 and 35 kDa), and sIL-20R2 (24 and 26 kDa) in the first peak, free sIL-20R1 in the second peak, and free sIL-20R2 in the third peak.

were treated with each ligand with increased amounts of soluble heterodimer sIL-20R1/sIL-20R2 or individual soluble receptors. The sIL-20R1/sIL-20R2 soluble receptor pair blocked luciferase activity stimulated by both ligands. sIL-20R1 alone was not capable of blocking the luciferase activity of any ligand, whereas sIL-20R2 alone blocked the activity of IL-19, although it did not have any effect on the activity of IL-20 at any concentration (*27*). We are not sure how to interpret the latter result, particularly since sIL-20R1 also did not affect the activity of IL-20, although one

possibility could be that sIL-20R2 affinity toward IL-20 is lower than that of the membrane-bound full-length IL-20R2. The specificity of interactions of sIL-20R2 with IL-19 was further confirmed in an additional binding assay, in which a soluble receptor was used to detect a ligand transiently expressed in COS-7 cells (*27*). We have shown above that free sIL-20R1 and sIL-20R2 do not interact with each other even at high concentration and thus cannot be expected to form heterodimers under physiological conditions. By extension, we assume that similar heterodimers are unlikely to be formed on the cell surface by full-length receptor molecules and the most probable sequence of events that leads to signaling involves creation of the ternary complex also involving a cytokine.

There was an attempt to generalize the identification of the high-affinity receptor chain (*29*) based on the crystal structure of the IL-10/sIL-10R1 complex (*30*) and on modeling of the IL-22/sIL-22R1 complex (*29*). It was suggested that residues Gly-44 and Arg-96 belonging to loops L2 and L4 of the receptor that interact with the ligand are largely responsible for the high affinity of the receptors in which they are present. Conversely, it was assumed that receptor molecules lacking these specific residues could only function as low-affinity receptors. Gly-44 and Arg-96 are conserved in IL-10R1, IL-22R1, IL-22BP, and IL-20R1 but are not present in either IL-10R2 or IL-20R2 (*29*). The data presented here suggest that even though these residues might be crucial for providing high-affinity binding in the IL-10 and IL-22 systems, this observation should not be extrapolated to the IL-19 and IL-20 signaling complexes. The binding studies presented here demonstrate that sIL-20R2 represents the high-affinity receptor for IL-19 and IL-20, whereas sIL-20R1 is a low-affinity receptor.

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