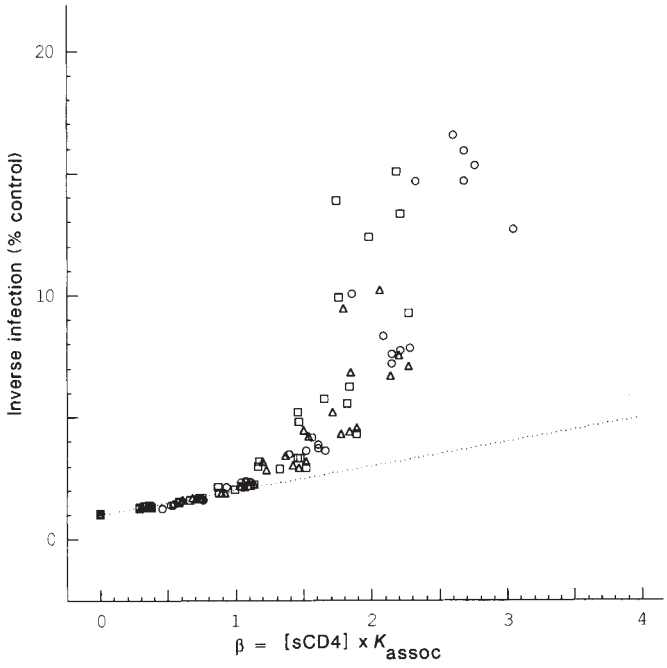

FIG. 3 Analysing the cooperative blocking activity of sCD4. Dividing inverse infection (Fig. 2) by its control (inverse infection without blocker) and multiplying sCD4 concentrations by the apparent K_{assoc} yields a normalized plot of inverse infection. Each normalization used the K_{assoc} calculated from the same target cell concentration (Table 1). The fraction of gpl20 molecules that are either free or blocked by a particular concentration of sCD4 is given by $1/(1+\beta)$ and $\beta/(1+\beta)$ respectively, where $\beta = [sCD4] \times K_{assoc}$. Results at all six target cell concentrations for HIV-1_{HXB3} (○), HIV-1_{MN} (□) and HIV-2_{NIHZ} (△). The dotted line represents blocking based on independent and equivalent gp120s (refs 21, 22). When less than half the gp120 molecules are blocked, the points lie on the dotted line. When half the gp120 molecules are blocked, deviations indicating sCD4 blocking synergy begin to occur. The synergies are identical for each viral strain.

their gp120) will be more easily blocked, thus enhancing the cooperativity in Fig. 3. Another explanation is that there are allosteric interactions between gp120s on the viral coat. This, in contrast to the recruitment model, predicts that the cooperativity of aged viral stocks will be unchanged or even decreased.

The upward curvature of the inverse infection plot is apparent only after half of the gp120 binding sites are blocked. Thus, the HIV envelope is covered by a highly redundant number of gp120 molecules which act independently at low sCD4 concentrations. Unlike HIV, viruses such as polio and influenza are covered by interacting (metastable) capsid polypeptide subunits and interacting glycoprotein subunits, respectively, which present relatively few critical neutralization sites. When a fraction of these sites are blocked by neutralizing antibodies, a non-local transition in subunit orientation is induced that inactivates the virus[28–30]. This property may contribute to the humoral efficacy of vaccines against polio and influenza. Neutralizing HIV, however, seems to be fundamentally different. □

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Additional deletion in sex-determining region of human Y chromosome resolves paradox of X,t(Y;22) female

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WHETHER a human embryo develops as a male or a female is determined by the presence of the Y chromosome[1,2]. The sex-determining function lies entirely in interval 1A, inasmuch as most XX individuals with descended testes and normal male external genitalia carry this small region of the Y chromosome[3]. We have localized an essential part of the sex-determining function to a portion of interval 1A, on the basis of the discovery of a female with a reciprocal Y;22 translocation and part of 1A deleted at the translocation breakpoint[3]. Recently, a paradox has arisen with the report[4] of four partially masculinized XX individuals who carry only a portion of interval 1A—a portion that does not overlap the deletion in the X,t(Y;22) female. These recent findings imply that the sex-determining function lies in the portion of 1A present in the four XX intersexes and not in the portion deleted in the X,t(Y;22) female. To explain the X,t(Y;22) individual, it was proposed that she was female because of a chromosomal position effect[4] or delayed development of the gonadal soma[5]. Here we report that the X,t(Y;22) female has a deletion of a second portion of interval 1A—a portion corresponding closely to that present[4] in the XX intersexes. This resolves the apparent contradiction. Nonetheless, phenotype–genotype correlations suggest that two or more genetic elements in interval 1A may contribute to the sex-determining function of the Y chromosome. The X,t(Y;22) female

lacks the *ZFY* gene but does not exhibit the complex phenotype known as Turner's syndrome, arguing against the hypothesis[5] that ZFY is the Turner's syndrome gene on the Y chromosome.

We have previously cloned, by chromosome-walking, a 230-kilobase (kb) segment of the sex-determining region of the Y chromosome, including part of deletion interval 1A1, all of intervals 1A2 and 1B, and part of 1C (ref. 3). (Intervals 1A1 and 1A2 together constitute interval 1A; see Fig. 1). By continuing this walk, we cloned the remaining, distal portion of interval 1A1; the pseudoautosomal boundary[6] was crossed.

Various DNA sequences derived from the distal portion of 1A1 were then hybridized to Southern blots of genomic DNAs from individuals known or suspected to carry part but not all of the Y chromosome, including several XX males and XY females. In general, the results were concordant with results previously obtained with probes from proximal 1A1 and 1A2. With one exception, all individuals were either positive for all of interval 1A (both distal and proximal 1A1, as well as 1A2) or negative for all of interval 1A.

The exception was WHT1013, a female with a reciprocal Y;22 translocation. This X,t(Y;22) female was previously shown[3] to have a deletion of intervals 1A2 and 1B but otherwise to carry most of the Y chromosome, including proximal 1A1. To our surprise, we found that this X,t(Y;22) female lacks the most distal 50 kb of interval 1A1. For example, she carries an 8-kb Y-specific *Taq*1 fragment detected by probe pDP1226 (about 65 kb proximal to the pseudoautosomal boundary) but lacks a 5.5-kb Y-specific *Hin*dIII fragment detected by probe pDP1225 (about 10 kb proximal to the boundary). As shown in Fig. 1, this deletion divides interval 1A1 into intervals 1A1A (absent in the X,t(Y;22) female) and 1A1B (present in the X,t(Y;22) female). Thus, X,t(Y;22) female WHT1013 has deletions of two portions of the Y chromosome—intervals 1A2 and 1B, and interval 1A1A. On the normal Y chromosome, these two deleted segments are separated by interval 1A1B which measures 90 kb.

Because interval 1A1A is immediately proximal to the pseudoautosomal region, it seemed possible that the second deletion would extend into the pseudoautosomal region. The single X chromosome in the X,t(Y;22) female is of maternal origin and presumably carries an intact pseudoautosomal region. We wished to determine whether or not the X,t(Y;22) female had inherited a pseudoautosomal region from her father. Accordingly, we traced the transmission of various pseudoautosomal restriction fragment polymorphisms (RFLPs) in the family of the X,t(Y;22) female. We found that she inherited the distal portion of the pseudoautosomal region from her father. At pseudoautosomal locus *DXYS28*, for example, the X,t(Y;22)

TABLE 1 Transmission of pseudoautosomal RFLPs to the X,t(Y;22) female

Locus	Probe	Recombination with sex phenotype (%)	Ref.	Transmission from father	Transmission from mother
DXYS14	29C1	50	14,15	+	+
DXYS20	pDP230	50	16	+	NI
DXYS28	pDP411a	38	16	+	NI*
DXYS15	113D	32	15,17	+	+
DXYS17	601	14	15	−	+
MIC2	pDP1001 pDP1002	2	18,19	NI	NI
DXYS77	pDP320a	0	20	−	+

DNA probes detecting pseudoautosomal RFLPs were hybridized to *Taq*I-digested DNAs from X,t(Y;22) female WHT1013 and her parents. The two columns on the right indicate whether or not the X,t(Y;22) female inherited an allele from each of her parents. Loci are ordered from telomere to centromere. (Loci exhibiting more recombination with the sex phenotype during male meiosis are distal to loci exhibiting less recombination.) NI, not informative.

* The *DXYS28* typings (Fig. 1) are consistent with the X,t(Y;22) female having inherited her 1.3-kb allele from her mother.

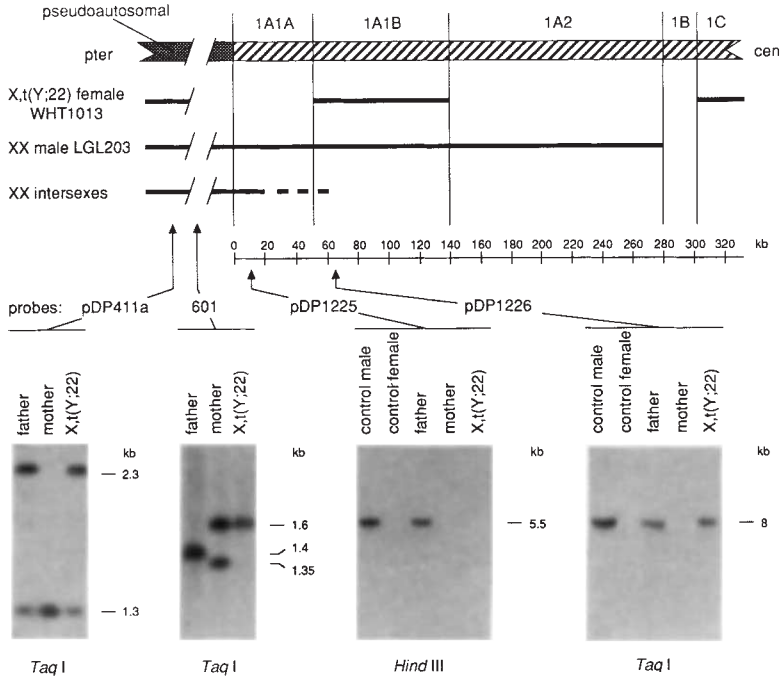

FIG. 1 Deletion analysis of X,t(Y;22) female WHT1013 by Southern blotting using pseudoautosomal and Y-specific DNA hybridization probes. Top, schematic representation of the distal short arm of the Y chromosome, oriented with respect to the telomere (pter) and centromere (cen). The pseudoautosomal region undergoes recombination with the X chromosome during male meiosis. Intervals 1A1A, 1A1B, 1A2, 1B and 1C show strictly sex-linked (as opposed to pseudoautosomal) inheritance and are defined by chromosomal deletions depicted immediately below. Black bars indicate the portions of the Y chromosome present in X,t(Y;22) female WHT1013 and in XX male LGL203 (ref. 3; case 6 in ref. 21). The stippled black bar indicates that the breakpoints in four partially masculinized XX individuals ('XX intersexes') fall somehwere between 20 and 60 kb proximal to the pseudoautosomal boundary[4], in interval 1A1A or 1A1B. Distances from the pseudoautosomal boundary[6] are shown in kilobases. Bottom, pseudoautosomal probes pDP411a and 601 and Y-specific probes pDP1225 and pDP1226 were hybridized to *Taq*I or *Hin*dIII digests of DNAs from X,t(Y;22) female WHT1013 and her parents. The sizes in kilobases of the hybridizing restriction fragments are indicated.

METHODS. Plasmids pDP411a and 601 have been described previously[15,16]. Plasmids pDP1225 and pDP1226 were subcloned from recombinant phages identified by extending a chromosomal walk[3] of interval 1A. Plasmid pDP1225 contains a 1.6-kb genomic *Hin*dIII–*Sal*I fragment subcloned into the *Hin*dIII and *Sal*I sites of Bluescript vector. (The *Sal*I site in pDP1225 derives from the phage vector and is not present in the human genome.) Plasmid pDP1226 contains a 1.0-kb genomic *Hin*dIII fragment subcloned into the *Hin*dIII site of Bluescript vector. Human genomic DNAs were prepared from lymphoblastoid cells, digested with restriction endonuclease, electrophoresed in 0.7% agarose, transferred to nylon membrane, hybridized at 47 °C with ^{32}P-labelled DNA probes, and exposed with X-ray film, all as previously described[3]. Before hybridization, radiolabelled inserts of pDP1225 and pDP1226 were preannealed with an excess of sonicated human genomic DNA[22].

TABLE 2 Absence of Turner's syndrome features in X,t(Y;22) female

Feature	Turner's syndrome	X,t(Y;22) female
Short stature*	+	−
Lymphoedema	Often	−
Webbing of neck	Often	−
Short fourth metacarpals	Often	−
Increased carrying angle of arms	Often	−
Coarctation of aorta	Often	−
Horseshoe kidney	Often	Normal right kidney Pelvic left kidney

* As compared with parental heights. At age 15 years 10 months, the X,t(Y;22) female's height was 164 cm (60th percentile).

female bears a 2.3-kb allele of paternal origin (Fig. 1, probe pDP411a). (The daughter's 1.3-kb allele is presumably of maternal origin.) But the X,t(Y;22) female did not inherit the proximal portion of the pseudoautosomal region from her father. For example, at *DXYS17*, the X,t(Y;22) female inherited a 1.6-kb allele from her mother but did not inherit the 1.4-kb allele for which her father is homozygous (Fig. 1; probe 601). As summarized in Table 1, the X,t(Y;22) female inherited an allele from her father for loci in the more distal portion of the pseudoautosomal region (*DXYS14*, *DXYS20*, *DXYS28*, and *DXYS15*), but she has no paternal allele for loci in the more proximal portion (*DXYS17* and *DXYS77*).

Thus, the Y chromosomal abnormality in the X,t(Y;22) female is complicated. Compounding the reciprocal Y;22 translocation is the deletion of two portions of the Y chromosome. The first deletion (of intervals 1A2 and 1B) is entirely within Y-specific DNA and is of 160 kb. The second deletion spans 50 kb of Y-specific DNA (interval 1A1A) but extends at least 600 kb and no more than 1,900 kb into the pseudoautosomal region (as estimated from physical maps[7,8]). Although we suspect that the complex deletion was created during the process of translocation, a firm understanding must await detailed analysis of deletion and translocation breakpoints.

The complex deletion in the X,t(Y;22) female is of immediate relevance to analysis of the sex-determining function of the Y chromosome. Most human XX males carry minute portions of the short arm of the Y chromosome and, though sterile, are otherwise masculinized, with descended testes and normal male external genitalia[9]. Such XX males are heterogeneous with respect to the amount of Y material they carry, but virtually all seem to carry the whole of interval 1A. Indeed, the smallest known segment of the Y chromosome in any such fully masculinized individual is interval 1A, as found in XX male LGL203 (Fig. 1 and ref. 3). Thus, all of the sex-determining function of the Y chromosome, whether one or several genes, maps to 1A (ref. 3). Four partially masculinized XX individuals[4] carry only a fraction of interval 1A (corresponding roughly to interval 1A1A; Fig. 1), indicating that a substantial portion of the sex-determining function of the Y chromosome maps to interval 1A1A. Having detected a seemingly simple deletion in X,t(Y;22) female WHT1013, we had previously concluded[3] that an essential portion of the sex-determining function occurred in interval 1A2. The finding of the second deletion (of interval 1A1A) in the X,t(Y;22) female undermines this previous conclusion but again emphasizes the importance of interval 1A, and especially of 1A1A, in sex determination. Given that four XX individuals[4] carrying only a small part of 1A are less masculinized than XX individuals carrying all of 1A, it may be that a gene or genes present in the intersexes[4] and a gene or genes in the remainder of 1A contribute to the sex-determining function of the Y chromosome. In any case, the female phenotype of the X,t(Y;22) individual is probably simply due to the absence of one or more sex-determining genes found in intervals 1A1A and 1A2.

The X,t(Y;22) female also sheds light on the aetiology of Turner's syndrome, a complex phenotype[10] often associated with a 45,X karyotype[1]. Turner's syndrome—or at least much of its somatic component— is apparently the result of monosomy for a gene or genes common to the X and Y chromosomes[11]. On the Y chromosome, these 'Turner's gene(s)' seem to be located in deletion intervals 1 or 2 (ref. 12; D.C.P. *et al.*, unpublished results). The *ZFY* gene lies in interval 1A2 of the Y chromosome[3] and has a closely related homologue, *ZFX*, on the X chromosome[13]. *ZFY* is deleted in the X,t(Y;22) female[3]. It has been suggested[5] that *ZFY* and *ZFX* may be Turner's genes. If this were the case, one would expect the X,t(Y;22) female, who has one copy of *ZFX* and lacks *ZFY*, to have some of the somatic features of Turner's syndrome. That she does not (Table 2) argues against *ZFX* and *ZFY* being Turner's genes. □

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Negative regulation of transforming growth factor-β by the proteoglycan decorin

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DECORIN is a small chondroitin–dermatan sulphate proteoglycan consisting of a core protein and a single glycosaminoglycan chain[1–3]. Eighty per cent of the core protein consists of 10 repeats of a leucine-rich sequence of 24 amino acids[2,4]. Similar repeats have been found in two other proteoglycans, biglycan[5] and fibromodulin[6], and in several other proteins including *Drosophila* morphogenetic proteins[7–11]. Expression of high levels of decorin in Chinese hamster ovary cells has a dramatic effect on their morphology and growth properties[1]. We now report that this effect is due at least in part to the ability of decorin to bind transforming growth factor-β, an autocrine factor[12,13] that stimulates the growth

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