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Development of a Somatic Mutation Screening System Using Hb Mutants.
IV. Successful Detection of Red Cells Containing the Human Frameshift Mutants Hb Wayne and Hb Cranston Using Monospecific Fluorescent Antibodies

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SUMMARY

The production and purification of antibodies detecting Hb Wayne, an α-globin frameshift mutant, and Hb Cranston, a β-globin frameshift mutant, are described. The antibodies are of a nonprecipitating nature, and they permit strong fluorescent labeling of erythrocytes containing Hb Wayne or Hb Cranston. Studies using artificial mixtures containing cells with either of the two mutants in frequencies ranging from 1 in 10^2 to 1 in 10^5 showed that fluorescent antibodies can detect rare mutant red cells in the presence of vast excesses of normal erythrocytes. On the basis of the structures and the molecular lesions underlying production of the two abnormal hemoglobins, we predict that the anti-Hb Wayne antibody will detect several frameshift mutants resulting from deletion of $3n + 1$ nucleotides or insertion of $3n + 2$ nucleotides at the 5′ side of the codon normally specifying residue 139 of the α chain. The anti-Hb Cranston antibody should be capable of detecting β chains, the corresponding genes of which have sustained insertions of $3n + 2$ nucleotides or deletions of $3n + 1$ nucleotides on the 5′ side of the codon normally specifying residue 144. The two antibodies may, therefore, prove

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to be valuable in the development of a system aimed at detecting rare erythrocytes that express mutations which arise in the hemopoietic stem cells of normal individuals and subjects exposed to mutagens.

INTRODUCTION

It is well established that several mutagenic substances exist in our environment and pose the risk of producing genetic damage to specific individuals, as well as to the population at large. Currently, efforts are underway to monitor the impact of environmental mutagens, using mutation-detection systems that are based primarily on exposure of lower organisms to the mutagens in question [1]. It has, however, become increasingly apparent that, in addition to these screening systems, mutation-monitoring systems aimed at detecting the effects of mutagens on man are required [1–8]. Efficient systems of this sort are not yet available, although Neel et al. [8] are attempting to use enzyme polymorphisms in field studies in Japan. Unfortunately, the monitoring of gametic mutations in man is cumbersome and requires the testing of large numbers of individuals in order to detect mutagenic effects [5]. More recently, the possibility of developing mutation-monitoring systems that are based on detection of mutant cells by the screening of large populations of human somatic cells has been raised [9–12].

The rationale underlying the screening of somatic cells for the effects of mutagens is straightforward. It is expected that, just as mutagens affect gametic cells and induce mutations, they also act on the somatic cells composing the various proliferating tissues of the body. Hence, in vivo, among the somatic cells of an individual, there must exist several that carry specific abnormalities resulting from spontaneous mutations as well as abnormalities deriving from the actions of environmental mutagens. If methods capable of detecting mutant cells through the screening of large cell populations were available, one might be able to determine mutation frequencies (and, with certain assumptions, mutation rates) in persons who have or have not been exposed to mutagens. Thus, somatic cell screening might permit monitoring for mutations by direct study of persons exposed to mutagens.

Previously, we outlined the possible advantages of such a system of screening for somatic-cell mutations, as well as certain requirements of this approach [13]. For example, since mutations underlying deficiencies of gene products can be mimicked by epigenetic phenocopies, only systems permitting the detection of structural alterations of a protein can be used for somatic-mutation screening [13]. For this reason, we have initiated an effort to establish methods that detect mutant hemoglobins in single red cells. These methods employ monospecific, fluorescent antibodies capable of detecting mutant red cells that are heterozygous for abnormal hemoglobins. We have previously described the development of methods for detecting Hb S [14] and Hb C [15] in single erythrocytes. The β^S chain is the product of a thymine→adenine transversion; the β^C chain results from a cytosine→thymine transition. We report here the development of antibodies that detect single red cells containing either the α-chain frameshift mutant,

Hb Wayne [16], or the β-chain frameshift mutant, Hb Cranston [17]. We also outline the reasons for which these antibodies will permit detection of a large number of α- or β-chain frameshift mutants that are not associated with molecular lethality or premature chain termination.

MATERIALS AND METHODS

The sources of Hb Wayne were two related A/Wayne heterozygotes [16], while red cells with Hb Cranston were obtained from the mother of the proband in whom Hb Cranston was initially detected [17]. The two abnormal hemoglobins were purified as described [16, 17].

Antibodies were raised in horses according to the immunization schedule described [14]. A total of 21,900 ml of serum was withdrawn (over a period of 1 year during which antigenic boosters were administered) from the one horse that responded to immunization with Hb Wayne. The maximum titer, ascertained by double immunodiffusion with Hb Wayne as antigen, was 1:64. The yields of sera from the three horses that were immunized with Hb Cranston were 12,600 ml, 10,500 ml, and 6,200 ml, respectively. One horse responded well (titer in double immunodiffusion plates, 1:32), while horses 2 and 3 responded poorly to the antigen (titers for one horse ranged from 1:2 to 1:8; for the other horse, 1:2); all of these sera were used in the effort to obtain anti-Hb Cranston antibodies.

Purification to the anti-Hb Wayne antibody followed published procedures [14, 15]. Briefly, approximately 1,000 ml of decomplemented sera were absorbed (at least twice) against Sepharose 4B (300–400 ml) to which the proteins in normal hemolysates had been covalently bound [18]. Total hemolysates (instead of purified Hb A) were used to absorb antibodies against any minor hemoglobins and nonhemoglobin proteins that might have contaminated the chromatographic preparations of Hb Wayne used for immunizations; failure to remove antibodies against such proteins would invalidate later attempts to detect the Hb Wayne-containing cells. To 3 vol of the Sepharose-hemolysate, 1 vol Sepharose-Hb A_3 (Hb A_3 having been isolated from normal lysates) was added. This was done because it is virtually impossible to completely separate Hb Wayne from Hb A_3 and, hence, removal of any anti-Hb A_3 antibodies during the purification process is necessary. To 3 vol of Sepharose-hemolysate + Hb A_3, 1 vol Sepharose-Hb F was also added to ensure removal of any anti-Hb F antibodies that might have been produced in response to Hb F molecules contaminating the preparations of Hb Wayne used for immunization. This mixture of Sepharose-hemolysate + Hb A_3 + Hb F will, subsequently, be referred to as Seph-Hb.

After absorption against Seph-Hb, the sera were absorbed against Sepharose beads to which purified Hb Wayne was covalently bound (Seph-Wayne). This absorption was accomplished by stirring 1 liter of sera with 300 ml of Seph-Wayne for 48 hrs at 4°C. The Seph-Wayne was subsequently packed in 5 × 10.5 cm columns, which were washed extensively before dissociation of the Hb-Wayne-antibody complexes with CO_2-saturated water (CO_2-H_2O). Since, during development with CO_2-H_2O, some hemoglobin was also eluted from the columns, contaminating the antibody preparation, further purification of the antibody was carried out using carboxymethylcellulose chromatography as described [14, 15]. Finally, the antibodies obtained from the columns were dialyzed against water, lyophilized, and conjugated with fluorescein isothiocyanate (FITC) [19]. Further procedures for purification of the anti-Wayne antibodies and monitoring of their specificity are described under RESULTS.

The purification of the anti-Hb Cranston antibodies followed the same steps described above for the purification of anti-Hb Wayne antibodies. Absorption against Seph-Hb (composed of hemolysate + A_2 + F) was followed by absorption against Sepharose-Hb Cranston. The Seph-Cranston was then packed in columns, and the antibodies were eluted with CO_2-H_2O. Separation of hemoglobin from antibodies was then accomplished in columns of CM-cellulose. After recovery by lyophilization and conjugation with FITC, the specificity of the antibodies was tested by applying the FITC-conjugates to mixtures of known proportions of A/Cranston and A/A cells.

The reaction of the fluorescent antibodies with fixed red blood cells was carried out as described [14, 15, 20], with the exception that the red-cell smears were fixed with ethanol-acetone (9:1 by volume) instead of methanol-acetone.

RESULTS

Anti-Hb Wayne Antibodies

Examination of the reactivity of the sera collected from the animals immunized with Hb Wayne, using double immunodiffusion, showed that they contained antibodies capable of recognizing all hemoglobins tested (Hb Wayne, Hb A, Hb F, Hb A_2); hence, the majority of the antibodies produced were directed against antigenic determinants that were common to these hemoglobins (fig. 1, *I*). However, after absorption against the Seph-Hb, no immunoprecipitation lines could be detected (fig. 1, *II*). Although tests of immunoprecipitation failed to demonstrate that the sera absorbed against Seph-Hb contained anti-Hb antibodies, the sera were further absorbed against Seph-Wayne. Elution of each Seph-Wayne column with CO_2-H_2O produced a definite peak, only a small proportion of which could be attributed to the hemoglobin that was released from the column (fig. 2*A*). After CM-cellulose chromatography, a definite antibody peak was obtained (fig. 2*B*). At this stage, a total of 435 mg of antibody was recovered from the 21,900 ml of serum. Double immunodiffusion, utilizing this purified antibody, failed to produce immunoprecipitation lines when Hb Wayne or normal hemoglobins were used as antigens (fig. 1, *III*).

A portion of the preparation obtained from the CM-cellulose columns was, after recovery by lyophilization, conjugated with FITC; labeled antibodies were then applied to fixed smears composed of 90% A/A cells and 10% Hb Wayne-containing cells. All of the red cells were labeled with the FITC-antibodies, but about 10% of the erythrocytes were more intensely labeled than the rest. Since all the red cells were labeled with the

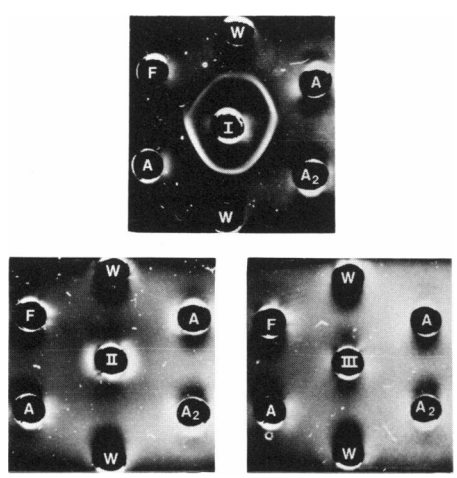

FIG. 1.—Double immunodiffusion plates showing reactivity of anti-Hb Wayne serum before and after purification. Central wells contained: *I*, original serum; *II*, serum after absorption against Seph-Hb (see MATERIALS AND METHODS); and *III*, antibody eluted from the Seph-Hb Wayne column and purified by CM-cellulose chromatography. Note absence of immunoprecipitation lines in *II* and *III*.

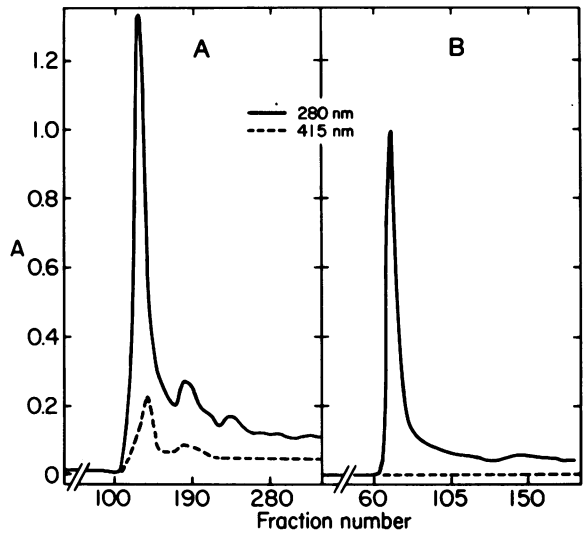

FIG. 2. —Elution profiles of: *A*, Sepharose Hb-Wayne column, showing a distinct protein peak; and, *B*, CM-cellulose column used for removal of hemoglobins that contaminated the antibody preparation. Absence in *B* of a peak at 415 nm indicates all hemoglobin remained bound to CM-cellulose.

FITC-antibodies, it seemed most likely that the preparation contained nonspecific, nonprecipitating, anti-Hb antibodies (hence, the negative reaction in figure 1, *II* and *III*). Since the proportion of Hb Wayne-containing red cells in the artificial mixture (i.e., 10%) matched the proportion of intensely labeled red cells, the antibodies recovered were judged enriched in specific anti-Wayne antibodies.

To remove the nonspecific anti-Hb contaminants, the lyophilized antibodies were dissolved and passed (twice) through columns of Seph-Hb, and the antibodies recovered from the columns were conjugated with FITC. Reaction of the anti-Wayne-FITC with an artificial mixture of Hb A/A (90%) and Hb Wayne-containing (10%) red cells produced strong fluorescent labeling of 10% of the cells and a uniform, but weak, labeling of the remaining erythrocytes. To remove the remaining nonspecific antibodies, the FITC-conjugated antibodies were again absorbed against Seph-Hb (24 hrs at 4°C with continuous stirring), concentrated, and reacted with mixtures of A/A and A/Wayne red cells. Figure 3 shows the labeling of an artificial mixture of A/A and A/Wayne red cells. It is obvious that only a proportion of red cells, those containing Hb Wayne, are labeled with the fluorescent antibody.

The results of experiments testing both the specificity and sensitivity of the anti-Hb Wayne-FITC in detecting Hb Wayne-containing cells are summarized in table 1. In these experiments, artificial mixtures of normal (A/A) and Wayne-containing erythrocytes were prepared and the smears of these mixtures were labeled with anti-Hb Wayne-FITC. Antibody-binding cells were then counted under fluorescent light, while the total numbers of cells in the same fields were counted under white light. Excellent correlation of the observed proportion of antibody-binding cells with the known proportion of Hb Wayne-containing erythrocytes was obtained.

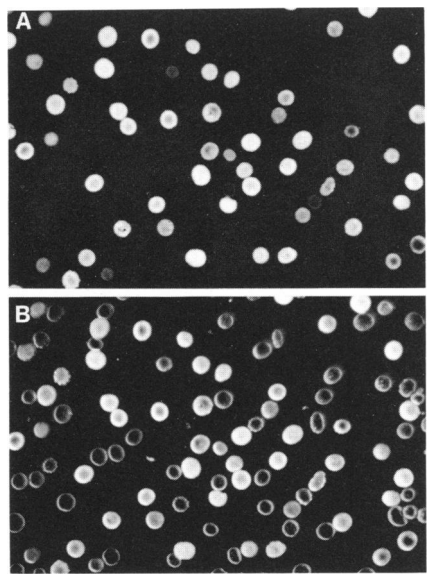

FIG. 3.—Reaction of an artificial mixture of normal (A/A) and Hb Wayne-containing (A/Wayne) erythrocytes with purified anti-Hb Wayne antibodies conjugated to FITC. *A*, Preparation is viewed through the FITC excitation beam and fluorescing cells are seen. Note heterogeneity in intensity of fluorescent labeling among the cells. *B*, Same microscopic field is simultaneously epilluminated with FITC beam and with transmitted white light; in addition to FITC-labeled Wayne cells, nonlabeled A/A cells are visible.

The anti-Hb Wayne-FITC was also used to label fixed preparations of cells from ten heterozygotes, each of whom had a different mutant hemoglobin; as expected, no signs of labeling were observed.

Anti-Hb Cranston Antibodies

The results obtained with the anti-Hb Cranston sera were essentially identical to those obtained with the anti-Hb Wayne sera. Specifically, the original serum reacted with the purified Hb Cranston as well as Hbs A, F, and A_2 (fig. 4, *I*). Absorption against Seph-Hb removed all the precipitating antibodies (fig. 4, *II*). The antibody, recovered after coupling with, and dissociation from, Sepharose-Hb Cranston, and further purification on CM-cellulose columns, also failed to produce immunoprecipitates (fig. 4, *III*). From the original stock of 29,300 ml of serum, 36.5 mg of anti-Hb Cranston antibody was recovered. This antibody, when conjugated with FITC and reacted with an artificial mixture of 90% A/A and 10% Hb Cranston-containing red cells, strongly labeled 10% of the erythrocytes. The remaining red blood cells displayed a weaker, homogeneous labeling, probably the result of contaminating anti-Hb A antibodies that had not been removed by previous purification. Absorption of the FITC-conjugated preparation against Seph-Hb A removed the nonprecipitating, cross-reacting anti-Hb A antibodies. The appearance of Hb Cranston-containing cells and of artificial mixtures of A/A and Hb Cranston-containing erythrocytes, after labeling with the anti-Cranston-FITC, are shown in figure 5*A* and *B*.

TABLE 1

OBSERVED AND EXPECTED FREQUENCIES OF FLUORESCENT CELLS IN ARTIFICIAL MIXTURES OF A/A:A/WAYNE CELLS LABELED WITH ANTI-HB WAYNE-FITC AND A/A:A/CRANSTON CELLS LABELED WITH ANTI-HB CRANSTON-FITC

Antibody used	Artificial mixture	Observed fluorescent cells	Expected Hb Wayne cells	Expected Hb Cranston cells
Anti-Hb Wayne-FITC	A/A cells:A/Wayne cells	0.78×10^{-2}	0.81×10^{-2}	...
		0.99×10^{-2}	" " "	...
		0.74×10^{-2}	" " "	...
		1.21×10^{-3}	0.88×10^{-3}	...
		1.30×10^{-4}	0.88×10^{-4}	...
		0.82×10^{-5}	0.88×10^{-5}	...
		1.25×10^{-5}	" " "	...
		0.75×10^{-5}	" " "	...
Anti-Hb Cranston-FITC...	A/A cells:A/Cranston cells	1.3×10^{-2}	...	1.3×10^{-2}
		1.5×10^{-3}	...	1.3×10^{-3}
		1.7×10^{-4}	...	1.2×10^{-4}
		1.3×10^{-5}	...	1.5×10^{-5}
		1.05×10^{-5}	...	" " "
		1.32×10^{-5}	...	" " "
		1.68×10^{-5}	...	" " "
		1.26×10^{-5}	...	" " "
		1.16×10^{-5}	...	" " "
		1.79×10^{-5}	...	" " "
		1.3×10^{-5}	...	" " "
		1.46×10^{-5}	...	" " "

Table 1 contains the results of experiments conducted to test the specificity and sensitivity of the fluorescent antibody. It is obvious that, down to a concentration of one Hb Cranston-containing cell per 10^5 A/A cells, the expected number of fluorescent cells was detected in the artificial mixtures. Thus, a specific antibody recognizing the abnormal β-chain sequence of Hb Cranston has been prepared. This antibody, like those previously raised against other mutant hemoglobins, was of a nonprecipitating nature.

DISCUSSION

There are several reasons underlying the use of abnormal hemoglobins in producing a system of value in screening for somatic mutations. Most important is the multitude of well-characterized, mutant hemoglobins. Over 300 abnormal hemoglobins, produced by single amino-acid substitutions, have been described. These hemoglobins result from transitions or transversions of single nucleotides in the genes encoding their chains. Antibodies against several of these mutants have been produced [14, 15, 21, 22], and fluorescent antibodies that permit detection of Hb S and Hb C have been prepared [14, 15] and used to detect, in normal subjects, the rare erythrocytes that appear to contain Hb S or Hb C [13]. Of the several hemoglobin mutants, of special interest are those containing elongated α or β chains: Hbs Constant Spring [23], Koya Dora [24], Icaria [25], Cranston [17], Wayne [16], and Tak [26]. The utility of antibodies specific for these mutants to a system for monitoring the occurrence of somatic mutations is based on consideration of the molecular defects underlying the production of elongated chains.

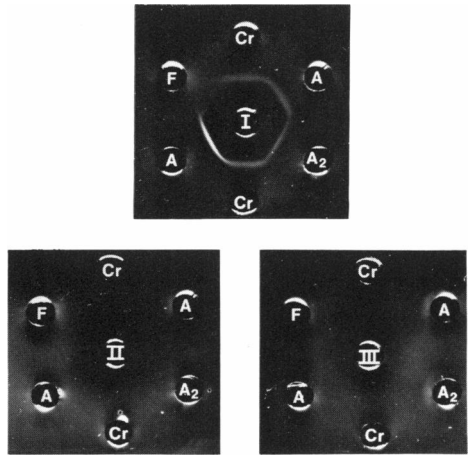

FIG. 4. —Double immunodiffusion plates showing reactivity of anti-Hb Cranston serum before and after purification. Central wells contained: *I*, original serum; *II*, serum after absorption against Seph-Hb (see MATERIALS AND METHODS); and *III*, final preparation of anti-Hb Cranston.

Hb Wayne is a mutant in which α chains are extended at the C-terminal end (table 2). This abnormality is ascribed to deletion of a base from the triplet that specifies either serine in position 138 or lysine in position 139 of the normal α chain. As a result, the reading of the mRNA shifts by one base and new codons are read until a new chain-termination codon is reached. Since the structure of Hb Wayne, with the

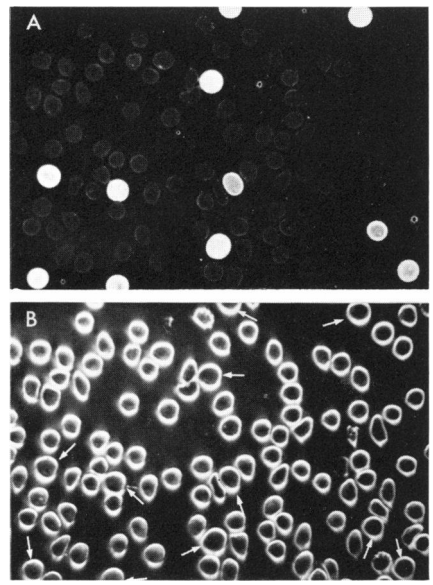

FIG. 5. —Reactions of an artificial mixture of normal A/A and Hb Cranston-containing cells with purified FITC-conjugated anti-Hb Cranston antibodies: *A*, field viewed through FITC filters; note brightly fluorescing cells; and, *B*, same field viewed in transmitted white light (without simultaneous FITC epillumination), showing all erythrocytes. Hb Cranston-containing cells are indicated by *arrows*.

TABLE 2

GENERATION OF THE NOVEL α^{Wayne} SEQUENCE BY VARIOUS INSERTIONS AND DELETIONS AT THE α-*Globin* GENE

Position in chain	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
α^A-chain sequence	---Val	Leu	Thr	Ser	Lys	Tyr	Arg	Stop								
α^A-mRNA*	5'---GUG	CUG	ACC	UCC	AAA	UAC	CGU	UAA	GCU	GGA	GCC	UCG	GUA	GCA-	---	---3'
α^{Wayne} mRNA	GUG	CUG	ACC	UC_C^A	AAU	ACC	GUU	AAG	CUG	GAG	CCU	CGG	UAG	CA-		
				[C] or [A] deleted												
Delete $3n + 1$, $n = 1$, bases†	GUG	CCU	CCA	AAU	ACC	GUU	AAG	CUG	GAG	CCU	CGG	UAG	CA-			
		[CUGA] deleted														
Insert $3n + 2$, $n = 1$, bases†	GUG	GGA	GCC	UGA	CCU	CCA	AAU	ACC	GUU	AAG	CUG	GAG	CCU	CGG	UAG	CA-
		[GGA GC] inserted														
Underlined base sequences all encode the C-terminal octapeptide							---Asn	Thr	Val	Lys	Leu	Glu	Pro	Arg	Stop	

* α^A-mRNA sequence data from [27, 28].

† Hypothetical cases of insertion and deletion.

exception of this C-terminal sequence, is identical to normal Hb A, the antibodies detecting Hb Wayne-containing cells do so by recognizing this abnormal sequence. However, the anti-Wayne antibodies will detect several other α-globin gene mutations that might arise through base deletions. Deletion of any one base from a codon on the 5′ side of that encoding position 139 will produce an α-chain frameshift; the sequence of the new α chain might differ from that of Wayne, but the C-terminal octapeptide will be identical to that found in Hb Wayne. A similar effect will be produced by deletion of any $3n + 1$ nucleotides (table 2). Similarly, insertions of $3n + 2$ nucleotides on the 5′ side of the codon specifying position 139 in the α-chain gene will produce a DNA sequence that will result in the production of a C-terminal octapeptide with the same sequence as that in the α-chain of Hb Wayne (table 2). Thus, it appears that an antibody detecting the extra C-terminal sequence of Hb Wayne will most probably detect all the α-chain frameshifts resulting from deletion of one (or $3n + 1$) bases or insertion of two (or $3n + 2$) bases 5′ to codon 139, provided that these mutations do not result in (1) premature chain termination (i.e., generation of a new termination codon within the normal bounds of the structural gene) or (2) molecular lethality (i.e., an unstable mRNA or α chain).

Similar considerations apply to the utility of the anti-Cranston antibody. It recognizes the novel tridecapeptide at the C-terminus of the β^{Cranston} chain. However, this tridecapeptide will be produced every time there is a deletion of one (or $3n + 1$) base or insertion of two (or $3n + 2$) bases on the 5′ side of the codon specifying the amino-acid residue in position 144 (see table 3). Provided that frameshifts in the β gene do not lead to molecular lethality or to premature termination, novel chain sequences will be produced that will contain the C-terminal sequence found in the β chain of Hb Cranston. The anti-Cranston antibody will thus provide the opportunity for detection of several β-gene frameshift mutations.

Antibodies have also been raised to Hb Constant Spring, an abnormal hemoglobin with elongated α chains. However, we have been unable to purify these antibodies, owing to the lack of sufficient antigen for preparation of Sepharose-Hb Constant Spring. The $\alpha^{\text{Constant Spring}}$ chains derive from a missense mutation of the terminating codon, resulting in the addition of an extra 31 amino-acid residues to the C-terminus. Antibodies against the abnormal portion of the Constant Spring sequence are expected to detect products of all missense mutations of the α-globin termination codon and, in addition, α chains produced by all deletions of $3n$ nucleotides that include the termination codon, provided they are not associated with molecular lethality.

The sensitivity of the antibodies produced is probably adequate for detection of these mutants, which may be present in low amounts in the red cell. Hb Wayne constitutes no more than 4%–6% of the total hemoglobin (1.1 to 1.6 pg per cell), yet it has been readily detected by the fluorescent antibodies we have produced (fig. 3).

Previously, we have used the fluorescent anti-Hb S and anti-Hb C antibodies to detect rare cells, which are labeled as are the Hb S- or the Hb C-containing red cells from AS or AC heterozygotes, in bloods from normal individuals [13]. By screening of fixed red-cell preparations under the fluorescent microscope, we found that, on the average, 1.1 in 10^7 red cells from normal adults are labeled as AS cells when exposed to anti-Hb S-FITC or as AC cells when exposed to anti-Hb C-FITC [13]. The time and

TABLE 3

GENERATION OF THE NOVEL $\beta^{Cranston}$ SEQUENCE BY VARIOUS INSERTIONS AND DELETIONS AT THE β-*Globin* GENE

Position in chain	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161
β^A-chain sequence	---Ala	His	Lys	Tyr	His	Stop														
β^A-mRNA*... 5′	---GCC	CAC	AAG	UAU	CAC	UAA	GCU	CGC	UUU	CUU	GCU	GUC	CAA	UUU	CUA	UUA	AAG	GUU	---	---3′
$\beta^{Cranston}$ mRNA ..	GCC	CAC	AAG	AGU	AUC	ACU	AAG	CUC	GCU	UUC	UUG	CUG	UCC	AAU	UUC	UAU	UAA	AGG	UU-	
				↑																
				[AG]	inserted															
Delete $3n + 1$, $n = 1$, bases†	GCC	AGU	AUC	ACU	AAG	CUC	GCU	UUC	UUG	CUG	UCC	AAU	UUC	UAU	UAA	AGG	UU-			
		↓																		
		[CACA]	deleted																	
Insert $3n + 2$, $n = 1$, bases†	GCC	GUG	UUC	ACA	AGU	AUC	ACU	AAG	CUC	GCU	UUC	UUG	CUG	UCC	AAU	UUC	UAU	UAA	AGG	UU-
			↑																	
		[GUG UU]	inserted																	
Underlined base sequences all encode the C-terminal tridecapeptide					---Ser	Ile	Thr	Lys	Leu	Ala	Phe	Leu	Leu	Ser	Asn	Phe	Tyr	Stop		

* β^A-mRNA sequence data from [29–31].

† Hypothetical cases of insertion and deletion.

effort required to visually screen several hundreds of millions of cells prohibited a study of the frequency of "Wayne-like" cells or "Cranston-like" cells in normal bloods. Procedures for detecting rare antibody-binding cells using electronic cell sorters are currently under investigation. The application of the monospecific antibodies we describe here for screening, using cell sorters, will permit study of the frequencies of Wayne-cells and Cranston-cells in normal individuals. Since several chemical mutagens, and radiation, produce frameshifts, it will be of special interest to examine the usefulness of our approach in monitoring somatic mutations by testing the frequency of these Wayne-cells and Cranston-cells in individuals exposed to the proper mutagens.

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