to human leukemia. The data presented show that not only is there significant sequence homology between v-*myb* and human c-*myb*, but that within human leukemic cells, the $p83^{c\text{-}myb}$ protein behaves like the leukemogenic $p45^{v\text{-}myb}$, in that it is localized to the nucleus and is associated with the nuclear matrix. This nuclear localization may be of particular interest, considering recent experiments demonstrating that differentiation of avian myeloblasts to macrophages is accompanied by relocation of the v-*myb* protein from the nucleus to the cytoplasm (*19*). This has been taken to imply that nuclear localization is important in maintaining cell transformation (*24*).

Comparisons of quantitative and qualitative changes in the c-*myb* transcript and/or gene product in normal and malignant cells may give insights into the role of this gene in human leukemia. Examples of both types of changes in proto-oncogene expression can potentially lead to cell transformation (*25, 26*). Of note in the current study is the marked increase, relative to the other patients, of c-*myb* transcripts in the two patients with AML from whom the cDNA clones were derived. All six patients examined had 95 to 100% leukemic blasts in their peripheral blood. Identical amounts of poly(A)$^+$ RNA from each patient were analyzed by Northern blot analysis. However, unlike the other four patients who had between 45,000 to 80,000 cells per cubic millimeter, these two patients had greater than 205,000 cells per cubic millimeter. Three other patients with similar levels of c-*myb* transcripts have been identified, and all have had peripheral blood leukemic blast counts in excess of 190,000 cells per cubic millimeter. It is tempting to speculate that the level of c-*myb* transcripts may correlate with or be related to the peripheral blood blast count; however, a larger survey of several patients will be required to address this. Correlation of other disease parameters with c-*myb* expression such as prognosis and response to therapy may also be possible in such a study.

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Structure, Antigenic Determinants of Some Clinically Important Insect Allergens: Chironomid Hemoglobins

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Determination of the molecular structure and properties of allergens that elicit severe immediate-type hypersensitivity diseases in humans and a knowledge of the structure of their antibody-binding sites should provide new insight into the pathogenetic mechanisms of allergic diseases. Monomeric and homodimeric hemoglobins (CTT I to X) have been identified as potent allergenic components of Chironomidae, a family of Diptera. Immunologic investigations of peptides of three of these hemoglobins (CTT IV, CTT VI, and CTT VIII) showed that human antibodies of the E and G classes recognize at least two different sites within each molecule. Individual hemoglobin peptides were aligned with homologous regions of chironomid hemoglobin CTT III, whose tertiary structure has been determined by x-ray analysis at a resolution of 1.4 angstroms. The antigenic site CTT IV(91 to 101) showed the following characteristics: (i) seven polar or hydroxylated amino acids, from a total of eleven, occupying predominantly superficial regions; (ii) the property of linkage to other molecules by hydrogen bonds or solvent clusters; and (iii) high thermal mobility factors. In contrast, peptide CTT IV(102 to 108), which does not bind human antibodies, contained no polar amino acids and had low thermal mobility factors. These results support the idea that the antigenicity of clinically relevant proteins is related to regions with a predominance of polar amino acids and with low energy barriers between different conformations, which allow high flexibility, including site-specific adaptation in antibody binding.

CONTACT WITH BOTH LARVAE AND midges of chironomids, nonbiting insects, elicits severe and immediate hypersensitivity in approximately 20% of environmentally or occupationally exposed persons (*1–3*).

A crude extract of larvae from one European chironomid species, *Chironomus thummi*, was fractionated (*1, 4–8*), and the fractions were studied by the radioallergosorbent test used to estimate allergen-specific antibodies of the immunoglobulin E class

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(IgE-RAST) in human serum. Eleven highly purified hemoglobins obtained after gel filtration and ion-exchange chromatography were found to be responsible for almost all of the antigenicity of the crude extracts. This relation could be demonstrated by applying the IgE-RAST method to sera of patients from different locations—for example, from the Nile region in the Sudan and from water-rich areas in the United States and Europe. Sequence analysis showed that all of these hemoglobins are composed of polypeptide chains with 136 to 151 amino acid residues, each complexed with one heme group (*4–9*). The molecular weights are approximately 16,000 and 32,000 for the monomeric and dimeric forms, respectively. The tertiary structures of these forms that were not cell-bound resemble those of vertebrate hemoglobins (*10*); each hemoglobin chain is folded in eight helical regions.

We studied the three chironomid hemoglobins CTT IV, CTT VI, and CTT VIII in detail. They were cleaved enzymatically and chemically; the fragments obtained were isolated and their antigenicity was assessed by the IgE-RAST method with sera from sensitized patients (Fig. 1). The following peptide sequences bound antibodies of the IgE class: from CTT IV, 1 to 90, 1 to 31, 32 to 90, 91 to 101, and 109 to 131; from CTT VI, 31 to 57, 38 to 140, and 64 to 98; and from CTT VIII, 1 to 65, 6 to 128, 66 to 100, and 129 to 139.

Hemoglobin CTT VI and its fragments were also studied for the binding of specific IgG antibodies in three persons clinically sensitized to chironomids. The binding pattern for IgG antibodies resembled that for IgE antibodies (Fig. 2). This nearly identical pattern suggests that human antibodies of both immunoglobulin classes react with the same antigenic determinants in the hemoglobin molecules.

CTT IV(91 to 101), a fragment with moderate antigenicity and a known three-dimensional structure, is composed of only 11 amino acid residues and is thus unlikely to contain more than one antigenic determinant (Table 1). The size of this peptide, however, exceeds the six-residue minimum for antigenic determinants apparently required for globular proteins, as shown for sperm whale myoglobin (*11*). Of the 11 amino acids, 7 exhibit polar or hydroxyl character. In contrast, the neighboring nonantigenic peptide CTT IV(102 to 108) (Table 1) has five hydrophobic amino acids and two amino acids with hydroxyl groups, but no polar amino acid. Antigenic sites consisting predominantly of polar amino acids have also been reported in sperm whale myoglobin (*11*), the α chain of human hemoglobin (*12*), soybean leghemoglobin (*13*), bovine and human serum albumin (*14, 15*), bovine

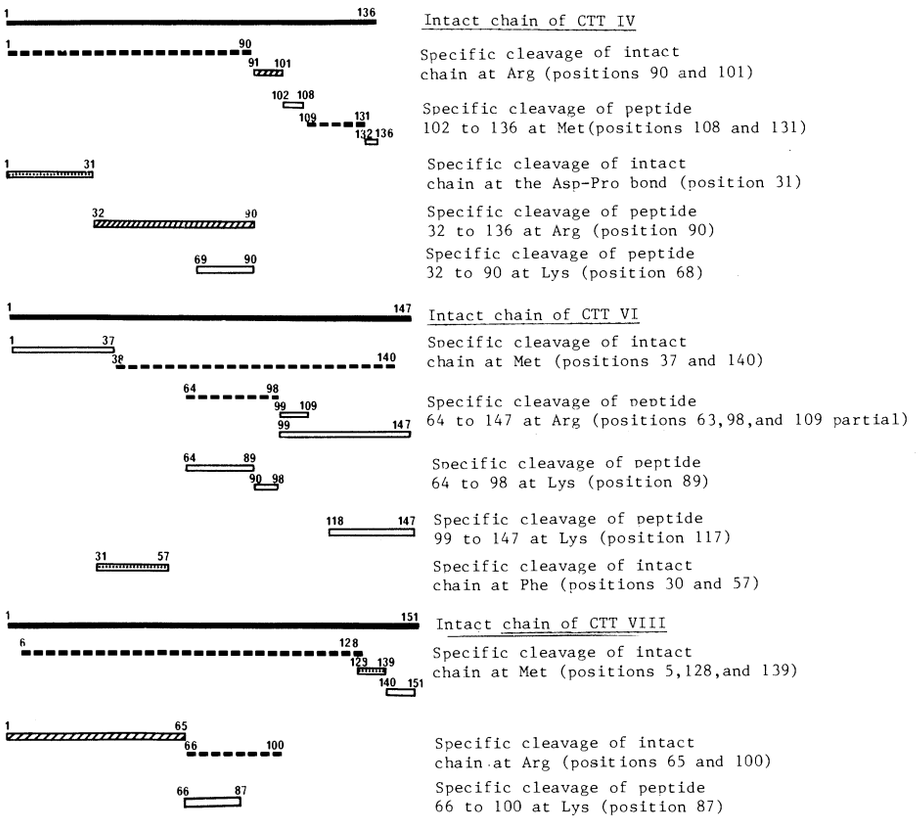

Fig. 1. Antigenic reactivity of fragments of three chironomid hemoglobins as evaluated by IgE-RAST. Hemoglobins CTT IV, CTT VI, and CTT VIII were cleaved by trypsin, cyanogen bromide, BNPS skatole, and formic acid, and the fragments thus obtained were isolated by gel filtration (on Sephadex G-75 and G-25 and on Bio-Gel P 6 and P 30), ion-exchange chromatography (on DEAE-cellulose, carboxymethyl cellulose, and Dowex 1-X2 and 50W-X4) and high-performance liquid chromatography (HPLC) (*1, 4–8*). The individual fragments were checked for purity by amino acid analysis, amino terminal analysis, or a combination of these. In all cases, impurities were below the limit of detection. The IgE-RAST method (*1, 23*) was used with commercially available reference standards (Phadebas-RAST; Pharmacia Laboratories, Uppsala) for evaluation of the antigenic reactivity of the native hemoglobins and their fragments. The sera used in the IgE-RAST originated from 12 patients who suffered from immediate-type hypersensitivity after being exposed to airborne components of chironomid larvae and who showed significant wheal and flare reactions in the skin test with *C. thummi* crude extract. The IgE-RAST values for the 12 patients were averaged for each hemoglobin and each fragment: ▭, no significant binding of IgE antibodies (≤3.4 U/ml); ▭, low (3.5 to 9.9 U/ml); ▨, moderate (10 to 29.9 U/ml); ■, high (30 to 100 U/ml); and ▬, very high binding of specific IgE antibodies (>100 U/ml). Test results were highly reproducible (coefficient of variation less than 10%). None of five unexposed atopic persons had an IgE-RAST value above 0.23 U/ml; control means + 2 SD were always below 0.35 U/ml.

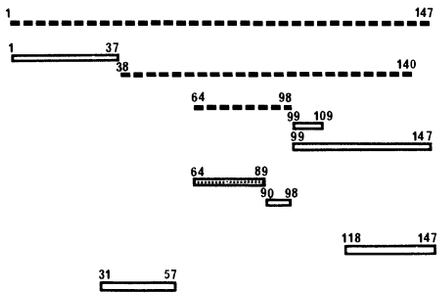

Fig. 2. Antigenic reactivities of fragments of hemoglobin CTT VI as evaluated by protein A-RAST. This modified RAST method (*24, 25*) differed from the usual procedure in that ^{125}I-labeled protein A instead of ^{125}I-labeled rabbit antibodies to human IgE was used. A serially diluted reference serum containing a high titer of specific IgG antibodies to the total hemoglobin fraction of *C. thummi* was used for construction of a standard curve. The amount of radioactivity bound, with 1 μl of this standard serum and *C. thummi* total hemoglobin used as the solid-phase antigen, was equal to 10 U/ml. Sera from three clinically sensitized patients, one of whom also had specific IgE antibodies, were investigated. The patterns of reactivity of these three patients were nearly identical. The RAST results obtained were averaged for the intact hemoglobin and each of its fragments: ▭, no significant binding of IgG antibodies (≤6.9 U/ml); ▭, low (7 to 9.9 U/ml); ▨, moderate (10 to 14.9 U/ml); ■, high level of specific IgG antibodies (≥15 U/ml). Test results were highly reproducible (coefficient of variation less than 12%). None of five unexposed atopic persons had an IgG-RAST value above 6.9 U/ml; control means + 2 SD were always below 6.9 U/ml.

Table 1. Amino acid sequences of the antigenic fragments CTT IV(91 to 101) and CTT IV(109 to 131), as well as of the nonantigenic fragment CTT IV(102 to 108). Capital letters indicate the location of the peptides within the helical structure (*9, 10, 22*).

Fragment	Peptide sequence
CTT IV(91 to 101)	^{91}Gly-Val-Thr-His-Asp-Gln-Leu-Asn-Asn-Phe-^{101}Arg FG G
CTT IV(102 to 108)	^{102}Ala-Gly-Phe-Val-Ser-Tyr-^{108}Met G
CTT IV(109 to 131)	^{109}Lys-Ala-His-Thr-Asp-Phe-Ala-Gly-Ala-Glu- G GH H Ala-Ala-Trp-Gly-Ala-Thr-Leu-Asp-Ala-Phe-Phe-Gly-^{131}Met H

fibrinogen (*16*), lysozyme (*17*), and influenza virus neuraminidase (*18*).

Atassi *et al.* (*11, 15*) concluded from their studies on five continuous antigenic determinants from sperm whale myoglobin that the binding of antibodies to antigenic sites is effected primarily through ionic interactions among polar amino acids, since modification of the polar residues within a binding region invariably destroyed its antigenicity. Intervening nonpolar and hydroxylated amino acids should provide stabilization through hydrophobic interactions and hydrogen bonding (*11*). The important role of polar amino acids might be explained by their presence in superficial protein regions where they are exposed to the solvent and thus accessible to antibodies.

Homologous sequences in the three hemoglobins studied and in the chironomid hemoglobin CTT III were aligned by computer (*9*). The tertiary structure of CTT III has been determined by x-ray analysis at a resolution of 1.4 Å (*10*). The three peptides from CTT IV were studied because the primary structures of two of them are identical to corresponding parts of CTT III (positions 91 to 101 and 102 to 108), and the third peptide (position 109 to 131) differs in only one amino acid, namely at position 127 (where in CTT IV, Ala is present, whereas in CTT III, Thr is present) (Table 1).

Using the three-dimensional crystal structure of the native hemoglobin CTT III as a reference, we found that the antigenic peptide CTT IV(91 to 101) is situated on the bend between helices F and G (Gly^{91} and Val^{92}) and at the beginning of the helix G (Thr^{93} to Arg^{101}). Some amino acids, namely Gln^{96}, Leu^{97}, and Phe^{100}, are involved in interatomic heme contacts (*10*). In addition, side chains of polar and hydroxylated amino acids are located on the surface of the molecule, where they make contact with solvent molecules: Thr^{93}, His^{94}, Asp^{95}, Asn^{98}, Asn^{99}, and Arg^{101}. In the crystal structure, these amino acid side chains are linked to neighboring protein molecules either by direct hydrogen bonds or by solvent clusters. The nonantigenic peptide CTT IV(102 to 108) is located within the G helix (G10 to G16). Only one amino acid side chain, Ser^{106}, forms hydrogen bonds with solvent molecules.

Similarly, by comparison with the known three-dimensional structure of CTT III, all other antigenic fragments of the three hemoglobins investigated seem to be in exposed conformational locations, for example, terminal sequences of helices or interhelical segments. However, our results suggest that not all surface structures are antigenic. Several other fragments containing such regions did not bind human antibodies, such as peptides CTT IV(69 to 90), CTT VI(1 to 37), CTT VI(99 to 109), CTT VI(99 to 147), and CTT VIII(66 to 87).

According to the results of Westhof *et al.* (*19*) and Tainer *et al.* (*20*), the antigenicity of superficial protein structures may be augmented by their high segmental mobility, that is, their high temperature factors. The sequence segment CTT IV(91 to 101) is not only located between two helices (F and G) and at the beginning of a helix (G), but is also a highly mobile region (*10, 21*). The following amino acid side-chain atoms have high thermal mobility (Fig. 3): His^{94}, Asp^{95}, Leu^{97}, Asn^{98}, and Asn^{99}. The values were calculated from x-ray crystallographic temperature data. Another antigenic peptide, CTT IV(109 to 131) (Table 1), also contains high thermal mobility at its initial region, which corresponds to the terminal sequences of helices G and H and to the interhelical bend GH: Lys^{109}, Asp^{113}, Ala^{115}, and Glu^{118} (Fig. 3). In comparison, the nonantigenic peptide CTT IV(102 to 108) has on average much lower thermal mobility, particularly for the side-chain atoms (Fig. 3).

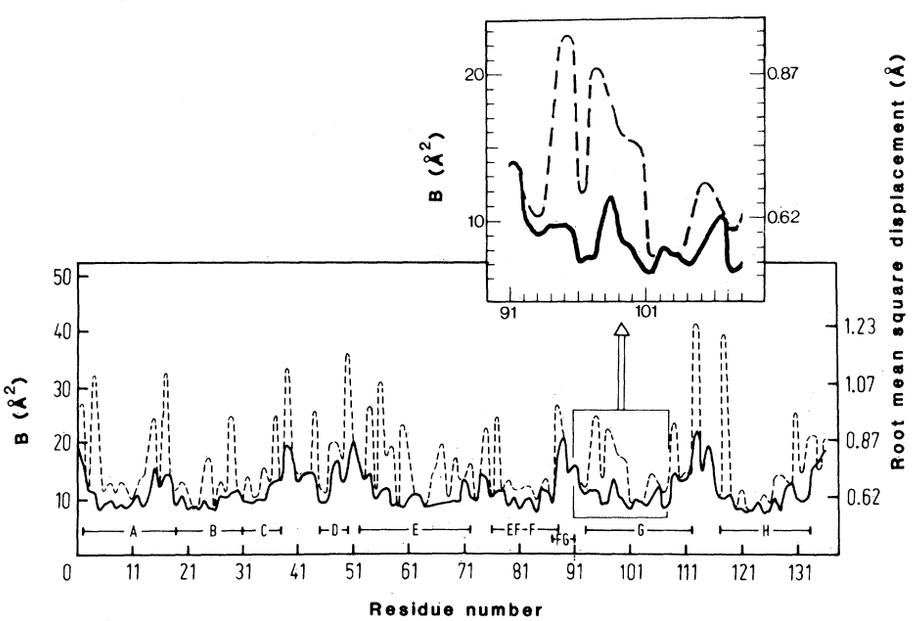

Fig. 3. Mean temperature factors (B) of the main- (———) and side-chain (·····) atoms plotted against residue number of hemoglobin CTT III. These data were derived from crystallographic refinement at 1.4 Å resolution (*10, 21*). From the crystallographic temperature factor B, the root mean square displacement is given by $\sqrt{3B/8\pi^2}$, which indicates the thermal mobility in angstrom units. The bars with the letters A to H indicate the helical regions (α or 3_{10}) of the CTT molecule along the residue axis.

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Differential Reflex Activity Determines Claw and Closer Muscle Asymmetry in Developing Lobsters

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The paired claws and closer muscles of the lobster, *Homarus americanus*, are identical in the early juvenile stages, but subsequently differentiate into a stout crusher claw with only slow fibers and a slender cutter with largely fast fibers. Rearing with different substrates or exercise of the claws revealed that claw laterality is determined in the central nervous system by differential reflex activity in the paired claws; the side with greater activity becomes the crusher, while the contralateral side becomes the cutter.

In many animals, asymmetry in an otherwise bilaterally symmetrical body plan often results in a specialization of function on opposite sides. A striking example of such asymmetry is seen in the paired claws of the lobster, *Homarus americanus*, where one of the claws is a stout, slow-acting crusher and the other is a slender, fast-acting cutter (*1*), each with its role in food collection and territorial defense (*2*). The muscular basis for this functional asymmetry resides in the fiber composition of the paired closer muscle; the cutter has 60 to 80 percent fast fibers and a small ventral band of slow fibers, and the crusher has 100 percent slow fibers (*3*). The crusher claw appears with equal probability on the right or left side of the animal (*4*); this suggests that claw laterality is randomly determined. Such determination occurs during early juvenile development, in the fourth and fifth stages (*5*). Prior to this the paired claws and closer muscles are symmetric; both look like cutters and have a central band of fast fibers sandwiched between slow fibers (*6*). Once claw laterality is determined in the early juvenile stages, the central fast fiber band becomes slow fibers in the crusher, while in the cutter the slow fibers change to fast, except for a small ventral band. Because claw laterality can be experimentally altered by manipulating the environment or the claws in the early juvenile stages (*5*), it may be possible to uncover mechanisms that govern asymmetry. Here we report such experiments, which demonstrate that differential reflex activity in the paired claws converges in the central nervous system (CNS) and determines claw and closer muscle asymmetry in juvenile lobsters.

Experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts, where the rearing facilities enabled us to follow the development of individual lobsters from hatching to late juvenile stages when their claws were clearly asymmetric. After hatching, the first three larval stages were reared communally. After the molt to the fourth stage, which is the first juvenile stage, each animal was transferred to an individual tray so that we could monitor its molt history and to prevent internecine behavior (*7*). Experimental treatments were carried out in the fourth and fifth juvenile stages because, during these 2 to 3 weeks, claw laterality is determined (*5*) and, once determined, remains fixed for life. Juvenile lobsters were reared to at least the eighth stage, when claw asymmetry is well established. At this stage the cutter claw was long and slender, and had distinctive sensory bristles and a central incisor-like tooth on its pollex; the crusher claw was short and stout, lacked the sensory bristles, and had a central molar-like tooth (Fig. 1). The cutter closer muscle clearly had the adult fiber composition of predominantly fast muscle with a ventral slow band, while the crusher muscle had not acquired its final composition of 100 percent slow fibers and still retained a narrow central fast fiber band (Fig. 1). Because muscle composition is closely tied to external claw morphology (*8*), the external morphology was utilized to assess the internal muscle composition. The fiber composition of the closer muscle was evaluated histochemically in three representative lobsters from each experimental condition, each with a different configuration of its paired claws (Table 1). Fiber analysis was performed on frozen cross-sections of the claw muscle that were stained for myofibrillar adenosine triphosphatase (ATPase) activity (*9*).

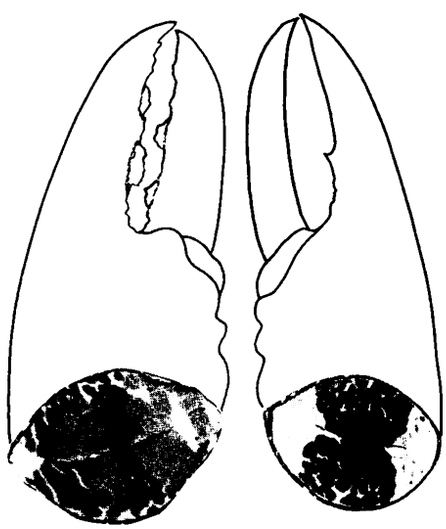

Fig. 1. Closer muscles of a left crusher claw and right cutter claw from an eighth stage juvenile lobster in which the left claw was exercised (magnified ×24).

When juvenile lobsters are reared with various substrates (such as oyster chips, plastic chips, gravel, or mud), as adults they have asymmetric claws and closer muscles in approximately equal numbers of right- and left-handedness (*8*). Such a ratio is to be expected because claw laterality is randomly determined (*4*). When they are reared without a substrate, however, they have symmetrical claws with closer muscles of the cutter type. In contrast to the substrates in the

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