Head Segmentation in Early Cambrian *Fuxianhuia*: Implications for Arthropod Evolution

Jun-yuan Chen, Gregory D. Edgecombe, Lars Ramsköld,*
Gui-qing Zhou

The arthropod *Fuxianhuia* from the Chengjiang fauna displays primitive aspects of cephalic segmentation and trunk limb morphology that indicate a basal position within Euarthropoda. The cephalon consists of an eye-bearing sclerite that articulates with a head shield bearing antennules and subchelate appendages. Eye stalks, antennules, and subchelate appendages are proto-, deuto-, and tritocerebral limbs and organs, respectively. The anterior position of the eye-bearing sclerite parallels the embryonic origin of arthropod eye lobes. The head of *Fuxianhuia* includes the acron and one somite and is regarded as a protocephalon. The definitive head of arthropods may have fused separate eye-bearing and appendage-bearing sclerites.

Arthropods have constituted the largest animal group on Earth since metazoans diversified in the Early Cambrian, and much research is devoted to elucidating their origins. Anatomical and molecular evidence favors a monophyletic arthropod origin (*1*), but no consensus exists on their phylogeny, the origin of biramous limbs, or patterns of segmentation in the head. Soft body parts that were preserved in extinct taxa from ancient faunas provide crucial evidence of what morphologies and structures arthropods actually had, widening the arthropod spectrum. Fossil evidence suggests that at least some of the well-separated arthropod subgroups in the extant biota are crown groups to long stem lineages of extinct forms (*2, 3*). The evolutionary origin of arthropod features is illuminated by fossil taxa such as anomalocaridids (*4*), which have several derived characters of Euarthropoda but lack the derived characters of any extant subgroup. The lack of fossils that represent basal euarthropods contributes to the lack of stability of branch positions in cladistic analyses of the major groups of arthropods (*5*).

Most early arthropods with soft-body preservation have been described from the Canadian Middle Cambrian Burgess Shale fauna (*6*). Of the more recently discovered "lagerstätten" (*7, 8*), the oldest, most diverse, and best preserved is the Early Cambrian Chengjiang fauna (*9, 10*). This ~525-million-year-old fauna is of Atdabanian age and was discovered in 1984 (*11*) in Yunnan, south China. It is positioned near the end of the short phase [only 5 to 9 million years long (*12*)] of the exponential increase of metazoan diversity during which nearly all phyla and classes present today originated (*13*). The Chengjiang fauna is dominated by arthropods (*10, 14*) including many primitive forms.

The arthropod *Fuxianhuia* was briefly described (*15*) soon after the discovery of the Chengjiang fauna. In number of individuals it constitutes less than 0.3% of the collected metazoan fossils. Although the primitive appearance of *Fuxianhuia* was subsequently recognized (*16*), it was treated as an arthropod of undetermined affinities (*14, 15, 17*) until its biramous trunk limbs were discovered (*18*). The multisegmented tergites led to the interpretation (*18*) of *Fuxianhuia* as an aschelminth- or flatworm-like, pseudometeric animal below euarthropod level (*19*). We describe here the segmentation and appendages of the head and trunk of *Fuxianhuia* and discuss their relation to the long-standing debate on early arthropod evolution.

The dorsal exoskeleton of *Fuxianhuia* (Fig. 1) is composed of a short head shield, a trunk with two regions or tagmata here referred to as a thorax and abdomen, and posterior fins with a telson-like terminal spine. Trunk tagmosis is defined by the width of pleural folds and presence or absence of sternites; the 17 (occasionally 16) thoracic tergites have wide pleural folds and lack associated sternites, and the abdominal 14 tergites all bear sternites but have increasingly narrow paratergal lobes on the first three. The first thoracic tergite is short and very narrow, and the second to fourth tergites are each progressively wider (Fig. 2B) (*20*). Tergites 1 and 2 lie completely beneath the head shield, whereas the third is only partly overlapped by the head (Fig. 2, C and F).

Slightly displaced from the anteromedian margin of the head shield is a small, rounded subrectangular plate, which extends laterally into stalks with black, bulb-shaped ends (Fig. 2, A and C to F). These are similar to known euarthropod stalked lateral eyes. Each stalk carries two constrictions, possibly indicating segmentation (Figs. 2A and 3C). A short posterior extension toward the central part of the animal, underlying the anterior margin of the head shield, evidently represents an articulating device (Fig. 2C). The eye-bearing sclerite is slightly variable in position between specimens, which suggests a limited mobility of the sclerite relative to the head shield.

The first cephalic appendage is a sensorial antennule composed of about 15 short antennomeres (Figs. 2A and 3C) and a long, slightly thicker proximal podomere (Figs. 2E and 3B). It passes beneath the anterior margin of the head shield and evidently is attached above the anterolateral edge of a wide ventral plate (Figs. 2F and 3A). This anteroventral plate articulated to the ventral margin of the head shield (apparently along a suture) and short sutures

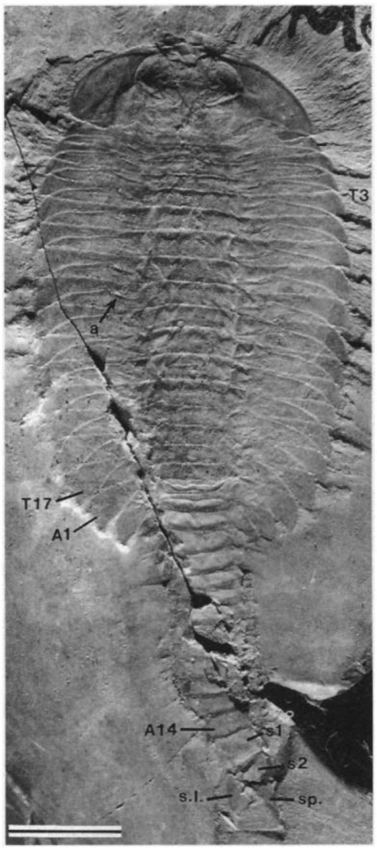

Fig. 1. Complete specimen ELRC 19255b of *Fuxianhuia protensa* Hou, 1987, from Chengjiang, exposed from the ventral side. Thoracic tergites 3 (T3) and 17 (T17), abdominal tergites 1 (A1) and 14 (A14), and the articulating device (a) at the anterior edge of T11 are indicated. Overlying (as preserved) the dorsal terminal spine (sp.) are an anterior sternite (s1) with the right side lobe (s.l.) and a posterior sternite (s2). For a close up of the head, see Fig. 2F; for a camera lucida drawing, see Fig. 3A. Scale bar, 1 cm.

J.-y. Chen and G.-q. Zhou, Nanjing Institute of Geology and Palaeontology, Academia Sinica, Chi-Ming-Ssu, Nanjing 210008, People's Republic of China.
G. D. Edgecombe, Australian Museum, Post Office Box A285 Sydney South, New South Wales 2000, Australia.
L. Ramsköld, Museum of Palaeontology, University of Uppsala, Norbyvägen 22, S-752 36 Uppsala, Sweden.

---

*To whom correspondence should be addressed.

define its anterolateral contact with a narrow doublure-like fold. Behind the short sutures, the anteroventral plate tapers backward; its rear margin is not well known but it appears to have been concave posteriorly.

The only postantennular cephalic appendages are a large uniramous pair that attach beneath the anteroventral plate (Figs. 2F and 3A). These are invariably flexed inward somewhat distal to the midlength of the appendage, indicating a single functional "knee." The general structure of the appendage is subchelate, with a very thick proximal part composed of at least three podomeres bearing one or more small spines laterally (Fig. 3, A and D). Distal to the knee the appendage is considerably more slender, and although it is divided into at least three podomeres (Fig. 3D), it is always preserved without curvature. It tapers to a blunt distal tip that is usually preserved as a black film indicating strong sclerotization (Fig. 2, A, C, and F). By analogy with crustaceans, for example remipedes (*21*), this sclerotization indicates that the tip of the appendage was its primary contact surface. The subchelate limb is the only identified mouthpart of the adult head in *Fuxianhuia*. The proximal podomere of the appendage lacks discernible subdivision into endites but bears numerous tubercles scattered on its lower surface (Fig. 3A), and an endite lobe (gnathobase) is apparently lacking. Preservation has affected the position of the subchelate appendages, but some specimens show that their proximal edges were capable of being brought nearly into contact (Fig. 2D). This, coupled with the single knee and sclerotized tip, indicates that food was manipulated by the tip and passed forward between the inner surfaces of the limbs to the mouth. No specimens preserve the mouth or gut in the head region, but a mouth opening beneath the anteroventral plate is consistent with the latter incorporating the labrum. The anterior edge of the first thoracic tergite is situated just behind the subchelate appendages (Fig. 2C), so it is highly unlikely that there would be space for additional cephalic appendages, and no traces of such have been observed.

There are two biramous appendages per tergite in the central part of the thorax, whereas on the one or two most posterior appendage-bearing tergites there may be three or possibly four biramous appendages (Fig. 4). There thus appears to be a disassociation between tergites and appendages. The endopod is parallel-sided with a rounded tip and consists of numerous (at least 15 in the central thorax, probably fewer posteriorly), weakly differentiated podomeres (Fig. 4C). The podomeres continue unchanged to the proximal end of the endopod; the limb base is cylindrical, longer than the endopod podomeres, and no endites have been observed. The anterodorsal endopod margin is smooth, whereas the posteroventral margin is serrated due to each podomere narrowing adaxially to permit some telescoping of the podomeres during posterior or ventral flexing of the appendage (Fig. 4C). The exopod lacks segmentation and is a large, rounded flap with a smooth, reinforced margin. Posterior exopods lack setae or spines, whereas in the central and anterior part of the thorax, numerous posterolaterally directed spines appear to be formed from folds of the upper exopod surface. The exopod is based on a short stretch of the anterodorsal side near the proximal end of the endopod; the details of its attachment are unknown.

Considerable softness of the exoskeleton of *Fuxianhuia* is indicated by commonly seen folds caused by compaction (Fig. 2, B and D). The softness may be partly due to decay after it was buried. A unique feature is a median fold in the head shield, extending from the anterior margin to about 80% the length of the head (Fig. 2, A and B). This structure may have provided some additional flexibility. There is a small occipital swelling near the posteromedian edge of the head shield (Figs. 2A and 3C) (*22*).

The thoracic tergites imbricate anteri-

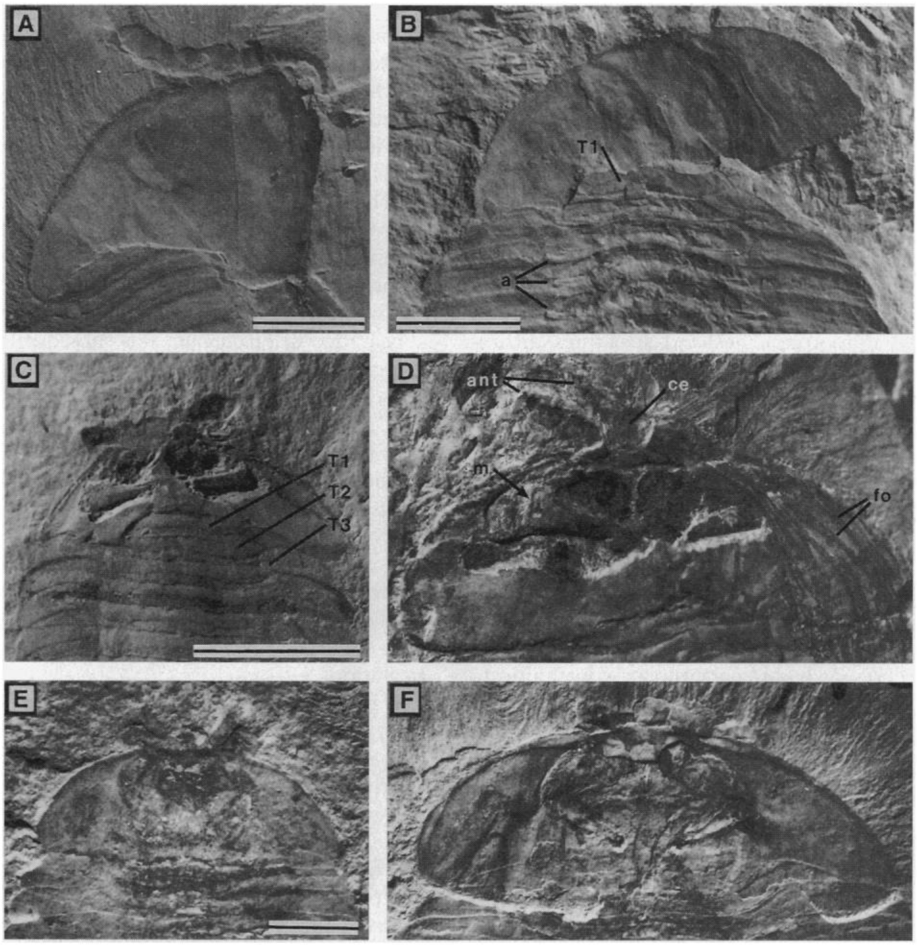

Fig. 2. *Fuxianhuia protensa* Hou, 1987, from Chengjiang. (**A**) Head of complete individual ELRC 19254a, dorsolateral view, with an outline of subchelate limbs impressed on the head shield. The right side is flexed down in matrix. See the camera lucida drawing in Fig. 3C for an explanation. (**B**) Head and anterior part of the thorax of complete individual ELRC19250a, dorsal view. The first thoracic tergite (T1) and the articulating devices (a) of T4 to T6 are indicated. (**C**) Head and T1 to T6 of holotype complete individual NIGP 100126a, dorsal view, after preparation of the subchelate limbs. The eye-bearing sclerite is complete. Tergite T4 is outlined by dark coloration. (**D**) Head of complete individual ELRC 19258, dorsolateral view, after preparation of the subchelate limbs. The outer margin (m) of the folded-over left limb is indicated with an arrow; the central part of the eye-bearing sclerite (ce), antennule (ant), and folds in the head shield (fo) are also indicated. (**E**) Head of complete individual ELRC 19251, dorsal view. See the camera lucida drawing in Fig. 3B for an explanation. (**F**) Head of complete individual ELRC 19255b, dorsal view, after preparation which revealed the posterior margin of the head shield (compare with Fig. 1). See the camera lucida drawing in Fig. 3A for an explanation. All scale bars, 5 mm; the bar in (A) applies also to (F), and the bar in (B) applies also to (D).

orly with a 30% overlap (Figs. 1, 2C, and 4D). The axial region forms a raised, transversely convex band, and the abdomen forms a continuation of this central trunk portion. A weak sagittal ridge is present on each tergite, running anteriorly from its rear margin and usually fading at midlength; the exoskeleton otherwise lacks sculpture. The abdominal sternites connect with the tergites laterally, producing a closed casing for the abdominal soft parts. The telson-like termination is composed of a large, dorsal part tapering posteriorly into a long, pointed spine. Dorsally it carries a posterodorsally directed fin. The sclerite is underlain by two sternites (Fig. 1). The anterior of these is subrectangular and carries posterolaterally a pair of smaller, stylet-shaped extensions. The posterior sternite is semicircular, and the sediment-filled gut in one specimen terminates at or near its posterior tip, beneath the posterodorsal spine.

In the axial region the thoracic tergites carry an articulating half-ring in front of a transverse articulating furrow. Lateral to the axial region the anterior tergite margin projects forward into a wide-based, conical extension (Fig. 4, B and D). Articulation between the tergites takes place along the adaxial side of the extension. Just behind the midlength (exsagittal) of the overlying tergite is an anterolaterally directed slit-like furrow (Fig. 2B) that articulates with a swelling (Fig. 1) on the anterior margin of the underlying tergite.

Some aspects of the morphology of the head shield in *Fuxianhuia* can be interpreted in terms of the function of its main appendage pair, the subchelate appendages. These differ greatly from the morphology of the biramous trunk limbs and appear to be a uniquely derived character of *Fuxianhuia*. Their inferred functional morphology indicates either a raptorial or a scavenging mode of feeding and is consistent with a grappling style of cephalic feeding being primitive for Arthropoda (*21*). The flexibility suggested by the median fold in the head shield may have been to accommodate limb movements. The streamlined body with tail fins and the stalked, anteriorly set eyes appear well suited for the active search or pursuit of prey. No gut contents have been found in adult *Fuxianhuia*, but several juvenile specimens show a gut filled with sediment indistinguishable from the surrounding matrix. This may either indicate ingestion of sediment during the death struggle when the individual was caught in the mud flow and entombed, or it may suggest normal feeding if the juveniles were deposit feeders and the adults were predators.

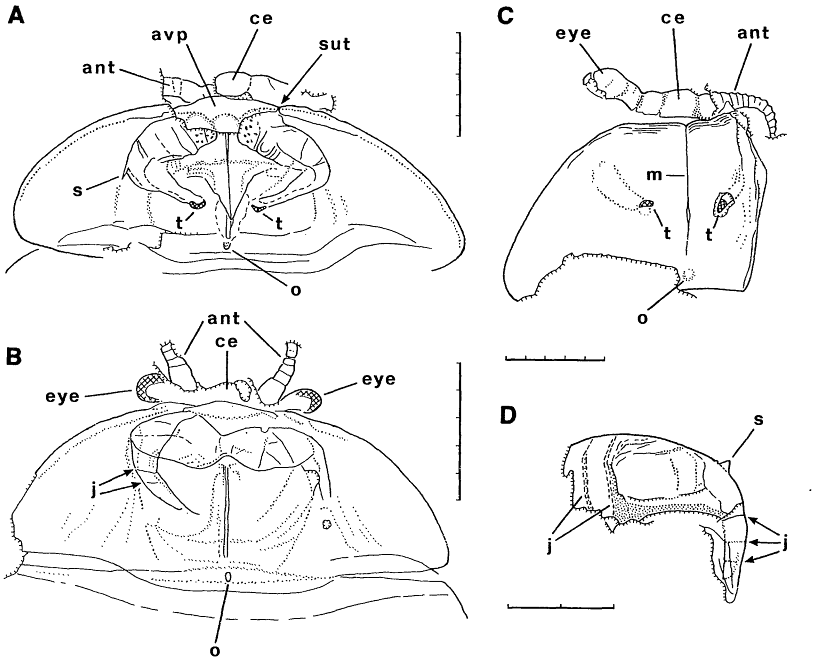

Fig. 3. Camera lucida drawings of the head structures in *F. protensa* Hou, 1987, from Chengjiang. Structures indicated are antennule (ant); anteroventral plate (avp); central part of the eye-bearing sclerite (ce); possible suture between the anteroventral plate and doublure-like fold (sut); spine (s), joints (j), and sclerotized tip (t) of the subchelate appendage; occipital structure (o); and median fold-like structure of the head shield (m). Scale bars in (A) to (C), 5 mm; scale bar in (D), 2 mm. (**A**) Head of complete individual ELRC 19255b, ventral view. Note the tubercles on the proximal part of the subchelate appendages. The sagittal line probably reflects the median fold of the head shield. (**B**) Head of complete individual ELRC 19251, ventral view. Note the large, basal podomere of the antennule. The cross-hatched area in the eyes is preserved as a dark film, interpreted as the visual surface. The raised area connecting the bases of the subchelate appendages is not part of the appendages. The double sagittal line is the median fold-like structure of the head shield. (**C**) Head of complete individual ELRC 19254a, oblique dorsolateral view. The right side is flexed down in the matrix. The sclerotized tips of the subchelate appendage were preserved almost in contact with the dorsal cuticle and were visible in dorsal view (see Fig. 2A). The camera lucida shows the specimen after initial preparation of the terminal part of the right side appendage (subsequent preparation exposed the complete appendages). The smooth occipital structure is raised. (**D**) Isolated subchelate appendage ELRC 19245. The most proximal part is missing. Dotted areas indicate deeply recessed areas.

Most important for the interpretation of primitive arthropod morphology is the development of the eyes in *Fuxianhuia* as organs of the anteriormost head somite. This position in the adult reflects a primitive condition otherwise detected in embryonic development. Throughout Arthropoda, the optic lobes are innervated by the protocerebrum, the anteriormost cerebral ganglia, whereas the first appendages (homologs of the crustacean antennules) are deutocerebral. This region, the archicerebrum or acron, is generally interpreted as being presegmental (*23*). The topological relations of the eyes, labrum, mouth, and antennae are typically modified through ontogeny (notably by forward shifts of the anterior appendages and rearward movement of the eyes) such that the fundamental order of somites and position of the acron are obscured in adults.

A labrum has not been observed in *Fuxianhuia*, but it is presumed to be incorporated into the anteroventral plate. By comparison with extant crustaceans and chelicerates (for example, anaspidaceans and xiphosurids), the anteroventral plate may be homologous with an epistome that bears the labrum posteriorly. This is consistent with the support of the first two pairs of cephalic appendages (antennules and subchelate limbs) by this plate. It is generally regarded that the ventral position of the labrum behind the antennules is secondary for euarthropods, with the labrum originating at the preantennular somite [possibly by fusion of an appendage pair at this somite (*24*)]. If the labrum is a component of the anteroventral plate in *Fuxianhuia*, this structure may be advanced to the same level as in other euarthropods.

The anterior eye-bearing sclerite of *Fuxianhuia* suggests that cephalization in Arthropoda involved a head with separate tergites that later were fused as a single unit. Such a style of cephalic tagmosis is observed in few other arthropods. An anterior cephalic sclerite bearing stalked eyes, antennules, and antennae in some crustaceans has been interpreted as being the simplest adult head of any arthropod and accordingly termed a protocephalon (*25*), although there is debate as to whether it is primitive or a secondary separation from the head (*26*). The definitive head is inferred to have formed by fusion of the anterior sensory sclerite (protocephalon) with the succeeding tergite associated with the mouthparts, the gnathocephalon. The eye-bearing sclerite of *Fuxianhuia* is superficially similar to the ocular plate of some crustaceans (for example, decapods) but differs in its anterior, rather than dorsal, position.

The primitive arthropods Euthycarcinoidea also have an anterior, sensory protocephalon bearing the eyes and antennules (*27*). Euthycarcinoids further resemble *Fuxianhuia* in diplo- and triplosegmentation of the trunk with decoupling of dorsal and ventral segmentation, the legless abdomen, and the numerous endopod podomeres, but they differ in having uniramous limbs and a single cephalic sclerite bearing eyes and antennules. Decoupled segmentation in primitive uniramous (euthycarcinoids) and biramous (*Fuxianhuia*) arthropods allows for the possibility that it is a general condition for Euarthropoda. Some support is provided by the irregular matching of dorsal and ventral segmentation in myriapods (*28*).

A head composed of the acron and at least one postacronal somite has been proposed as a ground plan character for Arthropoda (*26*). The definitive head of *Fuxianhuia*, composed of proto-, deuto-, and tritocerebral limbs and organs (the eye stalk, antennule, and subchelate appendage, respectively) is accordingly regarded as a protocephalon. Most extant taxa exceed this inferred basal condition in the adult, and a solely acronal head has been described only for the Cambrian *Sidneyia* (*29*).

For the origin and composition of the arthropod head in front of the second antennae, Snodgrass (*30*) posed the following question: Did the arthropod head ever consist of independently movable sections with a segmental musculature? Snodgrass postulated that in the absence of embryological data, convincing evidence of former segmentation in the embryonic cephalic lobe could only be provided by the discovery of a fossil arthropod with independent segments before the segment of the second antennae. We believe that *Fuxianhuia* provides an affirmative answer to this nearly 40-year-old question.

Several aspects of trunk limb morphology in *Fuxianhuia* appear to represent primitive conditions for Euarthropoda. These include the minimal differentiation of endopod podomeres and a lack of segmentation in the exopod. The lack of a gnathobasic endite lobe on the subchelate appendage as well as on thoracic limbs may be a primitive absence, implying a phylogenetic position below the most recent common ancestor of chelicerates and crustaceans. An inferred ground plan state of 8 to 11 podomeres in the euarthropod limb (*26, 31*) can now be interpreted as having been derived from an endopod with a larger number of divisions.

The basal position of *Fuxianhuia* within Euarthropoda indicated by trunk limb structure (*18*) is in accordance with the segmental composition of its head. Aspects of the trilobite-chelicerate-crustacean (TCC) style of grappling cephalic feeding in *Fuxianhuia*, such as midventral food transport to a posteriorly directed mouth, are reinforced as being primitive characters for Eurthropoda. Phylogenetic analyses of arthropods may now approach the problem of relationships between major clades, such as monophyly of Mandibulata versus a TCC group (*1, 5*), with character information from a primitive taxon with which more meaningful homology inferences can be made than with anomalocaridids or onychophorans.

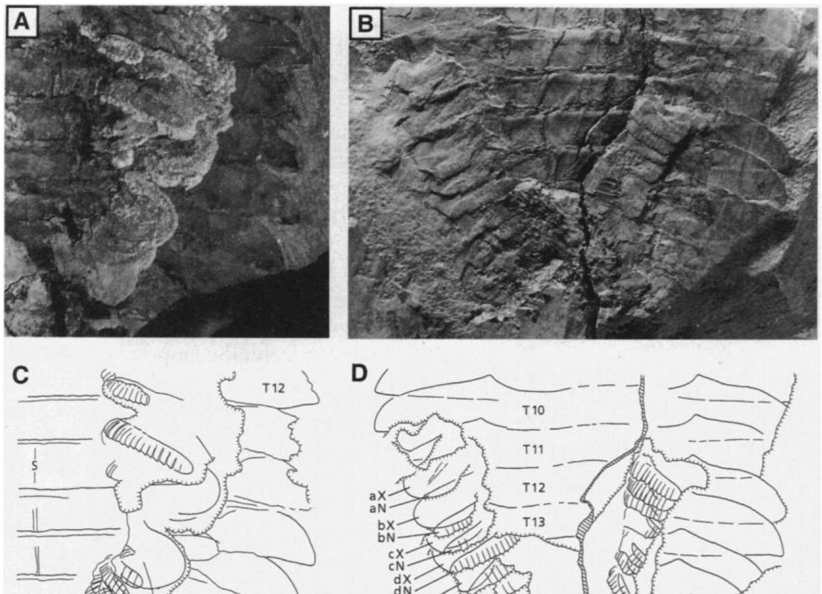

Fig. 4. Trunk limb structures in *F. protensa* Hou, 1987, from Chengjiang. (**A** and **C**) Detail and camera lucida drawings of specimen ELRC 19265a, dorsal view, prepared to show some limbs. Note the cluster of limbs associated with T16 and T17, and the large exopod flaps. The sagittal line (S) and first two abdominal tergites (A1, A2) are indicated. (**B** and **D**) Detail and camera lucida drawing of specimen ELRC 19264a, ventral view, showing limbs. Due to a slight lateral tilt of the specimen, the ventral structures are displaced to the animal's right side (left side as seen), with the right side limbs extended flat and the left side limbs curving to a vertical direction proximally. Eight consecutive biramous limbs of the right side are arbitrarily designated "a" to "h", with "X" denoting exopod and "N" endopod. Centrally on aX is a spine of the type more common anteriorly. The scale applies to all figures.

REFERENCES AND NOTES

1. J. W. Wagele, *Zool. Jahrb. Syst.* **120**, 253 (1993); W. C. Wheeler, P. Cartwright, C. Y. Hayashi, *Cladistics* **9**, 1 (1993).
2. D. Walossek, *Fossils Strata* **32**, 1 (1993).
3. L. Ramsköld, *Lethaia* **25**, 443 (1992).
4. J.-y. Chen, L. Ramsköld, G.-q. Zhou, *Science* **264**, 1304 (1994).
5. D. E. G. Briggs, R. A. Fortey, M. A. Wills, *ibid.* **256**, 1670 (1992); M. A. Wills, D. E. G. Briggs, R. A. Fortey, *Paleobiology* **20**, 93 (1994).
6. D. E. G. Briggs, D. H. Erwin, F. C. Collier, *The Fossils of the Burgess Shale* (Smithsonian Institution Press, Washington, DC, 1994).
7. S. Conway Morris, *Trans. R. Soc. Edinb. Earth Sci.* **80**, 271 (1989); ______, J. S. Peel, A. K. Higgins, N. J. Soper, N. C. Davis, *Nature* **326**, 181 (1987); M. F. Glaessner, *Alcheringa* **3**, 21 (1979).
8. R. A. Robison, in *The Early Evolution of Metazoa and the Significance of Problematic Taxa*, A. M. Simonetta and S. Conway Morris, Eds. (Cambridge Univ. Press, Cambridge, 1991), pp. 77–98.
9. J.-y. Chen and B.-D. Erdtmann, in (*8*), pp. 57–76.
10. X.-g. Hou, L. Ramsköld, J. Bergström, *Zool. Scr.* **20**, 395 (1991).
11. W.-t. Zhang and X.-g. Hou, *Acta Palaeontol. Sin.* **24**, 591 (1985).
12. S. A. Bowring *et al.*, *Science* **261**, 1293 (1993); C. E. Isachsen, S. A. Bowring, E. Landing, S. D. Samson, *Geology* **22**, 496 (1994).
13. J. J. Sepkoski Jr., in *The Proterozoic Biosphere: A Multidisciplinary Study*, W. J. Schopf and C. Klein, Eds. (Cambridge Univ. Press, Cambridge, 1992), pp. 553–561.
14. X.-g. Hou and J. Bergström, in (*8*), pp. 179–187.
15. X.-g. Hou, *Acta Palaeontol. Sin.* **26**, 272 (1987).
16. L. Delle Cave and A. M. Simonetta, in (*8*), pp. 189–244.
17. J.-y. Chen, J. Bergström, M. Lindström, X.-g. Hou, *Natl. Geogr. Res. Explor.* **7(1)**, 8 (1991).
18. J. Bergström, in *Lundadagarna i Historisk Geologi och Paleontologi 15–16 Mars 1993, III, Abstracts*, M. Siverson, Ed. (Lund University, Lund, 1993), p. 4.
19. Bergström's (*18*) statement of "roughly 3 pairs of legs throughout for each dorsal skeletal element" is not in accord with our findings. The suggested pseudosegmentation in *Fuxianhuia* (*18*) is rejected here together with the proposal that "segmentation

was invented among the arthropods after the invention of the exoskeleton."
20. The shortened, anteriorly narrowing trunk tergites of *Fuxianhuia* (Fig. 2B) are similar to a tagma that has been interpreted as the head in *Chengjiangocaris* (*14, 16*). We thus regard the sole specimen of *Chengjiangocaris* as a trunk for which the head shield is missing.
21. F. R. Schram and C. A. Lewis, in *Functional Morphology of Feeding and Grooming in Crustacea*, B. E. Felgenhauer, L. Watling, A. B. Thistle, Eds. (Balkema, Rotterdam, 1989), pp. 115–122.
22. The position of this swelling suggests comparison with the wide-spread "neck organ" or "dorsal organ" in other arthropods; see (*2*) for details.
23. The evidence as concerns the segmental nature of antennules has been disputed; F. H. Butt, *Biol. Rev. (Cambridge)* **35**, 43 (1960); S. M. Manton, *ibid.*, p. 265.
24. P. Weygoldt, in *Arthropod Phylogeny*, A. P. Gupta, Ed. (Van Nostrand Reinhold, New York, 1979), pp. 107–136.
25. R. E. Snodgrass, *A Textbook of Arthropod Anatomy* (Cornell Univ. Press, Ithaca, NY, 1952).
26. H. B. Boudreaux, in (*24*), pp. 551–586.
27. F. R. Schram and W. D. I. Rolfe, *J. Paleontol.* **56**, 1434 (1982). The hypothesis that euthycarcinoids are more closely related to hexapods than are myriapods [K. J. McNamara and N. H. Trewin, *Palaeontology* **36**, 319 (1993)] weights a character with a low consistency within Arthropoda (partially limbless trunk) and does not account for the absence of numerous atelocerate and mandibulate synapomorphies in euthycarcinoids. One recent analysis resolves euthycarcinoids as a sister group to all other euarthropods [F. R. Schram and M. J. Emerson, *Mem. Queensl. Mus.* **31**, 1 (1991)].
28. J. Bitsch, *Ann. Soc. Entomol. Fr.* **30**, 103 (1994).
29. D. L. Bruton, *Philos. Trans. R. Soc. London Ser. B* **295**, 619 (1981).
30. R. E. Snodgrass, *Smithson. Misc. Collect.* **138(2)**, 1 (1958).
31. J. Kukalová-Peck, *Can. J. Zool.* **70**, 236 (1992).

32. This study was supported by the Chinese Academy of Science and the National Geographic Society (grants no. 4760-92 and 5165-94). G.D.E. acknowledges support from the Australian Museum Trust. L.R. acknowledges support in China by the Wenner-Gren Foundation and Magn. Bergvalls Stiftelse.

10 November 1994; accepted 1 March 1995

Fibroblasts as Efficient Antigen-Presenting Cells in Lymphoid Organs

Thomas M. Kündig,*† Martin F. Bachmann, Claudio DiPaolo,
John J. L. Simard, Manuel Battegay, Heinz Lother,
André Gessner, Klaus Kühlcke, Pamela S. Ohashi,
Hans Hengartner, Rolf M. Zinkernagel*

Only so-called "professional" antigen-presenting cells (APCs) of hematopoietic origin are believed capable of inducing T lymphocyte responses. However, fibroblasts transfected with viral proteins directly induced antiviral cytotoxic T lymphocyte responses in vivo, without involvement of host APCs. Fibroblasts induced T cells only in the milieu of lymphoid organs. Thus, antigen localization affects self-nonself discrimination and cell-based vaccine strategies.

Efficient T cell activation requires two signals: "signal-1," received through the T cell receptor after engaging antigenic peptide on class I major histocompatibility complex (MHC) molecules, and "signal-2," a costimulatory signal. Originally proposed for B cells (*1*), this two-signal model is now regarded as a key mechanism in self-nonself discrimination for all lymphocyte subpopulations (*2*). Cells processing foreign antigens—professional APCs such as macrophages, dendritic cells, Langerhans cells, and B cells—would be the only cells that could provide costimulatory signals and therefore induce immune responses. Because all other cells of the body cannot provide costimulation, tissue-specific self antigens would not induce T cell responses, and autoimmunity would be avoided.

We now show that cells other than professional APCs can directly induce T cells. Mice injected with fibroblasts expressing viral proteins developed strong antiviral cytotoxic T lymphocyte (CTL) responses, without any involvement of professional APCs. Instead, class I MHC molecules on the fibroblasts were required for T cell induction. This result was unexpected, because the current model of T cell activation would predict that fibroblasts might anergize T cells, and CTL responses generated in these mice should have occurred only after antigen uptake and presentation of the fibroblast antigens by professional APCs (*2*). However, the additional finding that fibroblasts induced T cell responses only when they reached lymphoid organs may explain the discrepancy with other studies that showed that fibroblasts or tumor cells were unable to present antigen in an immunogenic manner (*3–5*).

The fibrosarcoma cell line MC57, which is derived from C57BL/6 (*H-2*^{b}) mice, was transfected with the glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV) to create a prototype of a nonprofessional APC (MC-GP) carrying a defined antigen (*6*). GP contains the dominant class I MHC epitope for CTL against LCMV in *H-2*^{b} mice [amino acids 33 to 41 presented on D^b and K^b (*7*)]. MC-GP cells expressed GP on the cell surface (Fig. 1A) and were susceptible to LCMV-specific CTL lysis (Fig. 1E). D^b expression was comparable to that on a lymphoma cell line (EL-4) (Fig. 1B). ICAM-1 and LFA-1, known to facilitate costimulation (*8*), were not detectable on MC-GP (Fig. 1C). MC-GP was also negative for expression of B7 molecules when stained with CTLA-4Ig (Fig. 1D) (*9*). A functional assay eliminated the possibility that MC-GP cells produced significant amounts of costimulatory cytokines. Unless exogenous cytokines were added, MC-GP cells could not restimulate memory CD8$^+$ T cells specific for LCMV in vitro, which have a minimal costimulatory requirement compared to naïve T cells (*10*) (Fig. 1E). Thus, MC-GP cells did not express costimulatory molecules and did not produce functionally significant amounts of costimulatory cytokines.

In initial studies we examined whether MC-GP cells could induce CD8$^+$ CTL responses against GP in vivo. Syngeneic C57BL/6 (*H-2*^{b}) mice were immunized intraperitoneally with MC-GP cells, and subsequent CTL responses against GP were assessed by means of in vivo and in vitro assays (Table 1, experiments A to C) (*11*). Mice immunized with MC-GP cells developed CTL-mediated protective immunity against lethal LCMV-induced choriomeningitis and were resistant to challenge infection with a vaccinia LCMV-GP recombinant virus, and LCMV-specific cytotoxicity could be restimulated in vitro. CTL-dependent in vivo protection was also induced in CD4$^+$ T cell–depleted mice (*12*) (Table 1, experiment C), suggesting that T help was not limiting. In summary, immunization with MC-GP cells induced strong GP-specific CTL responses.

We did the following experiments to examine the involvement of host APCs in CTL induction by MC-GP:

1) If host APCs were involved in the

T. M. Kündig, Institute of Experimental Immunology, University of Zürich, Schmelzbergstrasse 12, 8091 Zürich, Switzerland, and Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada.
M. F. Bachmann, C. DiPaolo, M. Battegay, H. Hengartner, R. M. Zinkernagel, Institute of Experimental Immunology, University of Zürich, Schmelzbergstrasse 12, 8091 Zürich, Switzerland.
J. J. L. Simard and P. S. Ohashi, Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada.
H. Lother, A. Gessner, K. Kühlcke, Heinrich-Pette Institute for Experimental Virology and Immunology, University of Hamburg, Martinistrasse 52, D-2000 Hamburg, Germany.

---

*To whom correspondence should be addressed.
†Present address: Ontario Cancer Institute, Department of Medical Biophysics and Immunology, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada.