phosphate (AMP) indicate that the N^6-(isopentenyl)-adenosine is coupled to 22-phosphoecdysone at carbon C-5′.

Finally, a large signal at $\delta = 65$ ppm in ^{13}C NMR spectra of the unhydrolyzed conjugate is assigned to C-5′-O-P junction; a similar observation was reported for the covalent bond between ribose and 22-phospho-2-deoxyecdysone in the 22-adenosine monophosphoric ester of 2-deoxyecdysone (*10, 11*). Our data indicate that the ecdysone conjugate present in newly laid eggs of *Locusta* corresponds to the 22-N^6-(isopentenyl)-adenosine monophosphoric ester of ecdysone. It is not surprising that this conjugate is hydrolyzed by the enzyme mixture of *Helix pomatia*, which is known to contain phosphodiesterase together with a large variety of other enzymes. Commercial phosphodiesterase (Sigma; purified) similarly cleaves this ecdysone conjugate as it cleaves the recently identified adenosine monophosphoric ester of 2-deoxyecdysone.

The result that the ecdysone conjugate is an isopentenyl-adenosine monophosphoric ester is unexpected and of great potential interest; N^6-(isopentenyl)-adenine (i^6A) is known as a cytokinin, that is, one of a family of substances which promote cell division and exert other growth regulatory functions in plants. The nucleoside N^6-(isopentenyl)-adenosine has been found in many plants as a free nucleoside and recently in mouse cells and in human cells as a free mononucleotide; C-5′ monophosphate derivatives of a related cytokinin ribosylzeatin have also been reported (*23–25*). According to Burrows (*25*), hydrolysis of the naturally occurring cytokinin ribosides increases their biological activity up to tenfold, mainly due to the removal of the ribose yielding the more active base.

From data obtained in *Locusta* eggs (*3, 26*), we know that the maternal ecdysone conjugate is hydrolyzed progressively during embryonic development. This hydrolysis yields the free steroid hormone ecdysone either directly, or via a first hydrolytic step cleaving at the C-5′-O-P junction to the intermediate 22-phosphoecdysone, a compound that is present during embryonic development of *Locusta* (*27*) and has also been reported from eggs of the related insect species *Schistocerca* (*28*).

The fate and the role in embryonic development of the cytokinin (i^6A) liberated on the process of hydrolysis of the maternal ecdysone conjugate appears as a challenging object of investigation. In this respect, the possibility should also be left open that the isopentenyl group could be used for the construction of the carbon skeleton of juvenile hormones (*29*). This report, and the recent identification of maternal AMP-2-deoxyecdysone (*10*) in newly laid eggs of *Locusta*, unravel an extremely elaborate strategy of the maternal organism for supplying its offspring with hormonal molecules which the embryo is obviously uncapable of synthesizing de novo before advanced stages of development.

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11 May 1982; revised 18 January 1983

A Glycolipid Antigen Associated with Burkitt Lymphoma Defined by a Monoclonal Antibody

Abstract. *The antigen defined by a rat monoclonal antibody directed to a Burkitt lymphoma cell line was identified as globotriaosylceramide [Galα(1 → 4)-Galβ(1 → 4)-Glcβ(1 → 1)-ceramide]. The antibody demonstrated a strict steric specificity since it did not react with globoisotriaosylceramide [Galα(1 → 3)-Galβ(1 → 4)-Glcβ(1 → 1)-ceramide], the positional isomer of the antigen associated with the Burkitt lymphoma. Chemical analysis of various Burkitt lymphoma cell lines revealed that the Burkitt lymphoma cells contained more than 100 times as much of the glycolipid antigen as was found in other human lymphoma and leukemia cell lines.*

A rat monoclonal antibody to a Burkitt lymphoma cell line (Daudi) was generated by hybridoma techniques (*1*). This antibody, designated 38-13, was found to be of the immunoglobulin M class; it defines an antigen specifically expressed on most malignant B-cell lines derived from Burkitt tumors, whether the lymphoma cells contain the Epstein-Barr virus (EBV) genome (Central-East African endemic type) or do not contain the EBV genome (European–North American type). The antigen was not detectable on EBV-positive lymphoblastoid cell lines, on normal or mitogen-activated lymphocytes, or on fresh malignant cells from patients affected with various lymphoproliferative disorders other than Burkitt lymphoma (*1, 2*). An earlier study indicated that the antigen was resistant to protease and soluble in a mixture of chloroform and methanol; it was presumed to be a glycolipid (*3*). In this report, we describe the isolation of the antigen and its chemical identification as globotriaosylceramide, which occurs in the erythrocytes of the rare blood group phenotype P^k and is called the P^k antigen (*4, 5*).

The glycolipid, which showed an in-

tense reaction with the 38-13 antibody in a solid-phase radioimmunoassay (*6*), immunostaining on thin-layer chromatography (*7*) (Fig. 1A) and a liposome lysis assay (*8*) (Fig. 1B), had the same mobility on high-performance liquid chromatography and high-performance thin-layer chromatography as a ceramide trihexoside (globotriaosylceramide) (*9*). The structure of the purified glycolipid was further confirmed by methylation analysis (*10*). (i) The permethylated glycolipid gave 2,3,4,6-tetra-*O*-methylgalactitol, 2,3,6-tri-*O*-methylgalactitol, and 2,3,6-tri-*O*-methylglucitol in an approximately equimolar ratio after acetolysis and reduction; 2,4,6-tri-*O*-methylgalactitol and any aminosugar derivatives were not detectable. (ii) The glycolipid was degraded and the reactivity with monoclonal antibody 38-13 was abolished by incubation with ficin α-galactosidase (*9, 11*) but not with jack bean β-galactosidase nor with jack bean β-*N*-acetylhexosaminidase (*9, 11*). (iii) A globotriaosylceramide (Gb3) (*12*) isolated from human erythrocytes (*9*) had the same TLC mobility as the glycolipid antigen isolated from three different Burkitt lymphoma cell lines (Fig. 2) and displayed the same degree of antigenic activity.

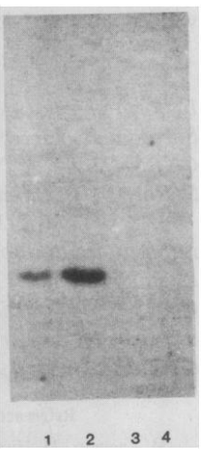


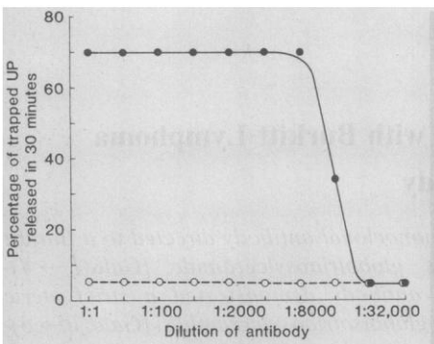
Fig. 1. The reactivity of ceramide trihexoside (globotriaosylceramide) with monoclonal antibody 38-13 to Burkitt lymphoma. (A) Immunostaining pattern of ceramide trihexoside by the antibody. Glycolipids were separated on a high-performance thin-layer chromatography plate (J. T. Baker Chemical Co.) with a mixture of chloroform, methanol, and water (100:40:6) and stained by a modification of the procedure of Magnani *et al.* (*7*). (Lane 1) Mixture of neutral glycolipids isolated from human erythrocytes; (lane 2) a purified globotriaosylceramide; (lane 3) gangliotriaosylceramide (Gg3); and (lane 4) lactotriaosylceramide (Lc3). The glycolipid fraction was obtained as follows: Packed cells (8 to 10 ml) were extracted and purified through a modification of Folch's partition (*30*), an acetylation procedure (*31*), and DEAE-Sephadex chromatography (*32*). The antigen was further purified through high-performance liquid chromatography according to a modification (*33*) of a procedure described by Watanabe and Arao (*34*) with an Iatrobeads RS8010 (10-μm beads) column (4 mm by 30 cm) (Iatron Chemical Co., Tokyo). Gradient elution was performed with a mixture of isopropanol, hexane, and water (55:40:5 to 55:25:20). A total volume of 40 ml was collected in 80 fractions. Elution of glycolipid references occurred as follows: ceramide monohexoside, fractions 6 to 8; ceramide dihexoside, fractions 9 and 10; ceramide trihexoside, fractions 14 to 18; and globoside, fractions 23 to 25. The major antigenic activity was concentrated in fractions 14 to 18, which contained both glycolipid and fluorescamine-positive material. The pooled fractions were separated on high-performance thin-layer chromatography. Bands containing fluorescamine-positive and orcinol-positive material were removed and extracted with a mixture of isopropanol, hexane, and water (55:20:25). The glycolipid fraction contained the antigenic activity that was analyzed by immunostaining.

Only Burkitt lymphoma cell lines contained a large quantity of the glycolipid (200 to 800 μg per 100 mg of dried cell residue). The human B lymphoblastoid cell line Priess, the T cell line JM, and the erythroleukemia cell line K562 (*13*) did not contain this glycolipid (see Fig. 2).

The specificity of the antibody was tested by comparing complement-mediated lysis of liposomes (*8*) containing other glycolipids with a similar structure. Globoisotriaosylceramide (rat kidney ceramide trihexoside) (*14*) and rabbit ceramide pentasaccharide (*15*), which both have a Galα(1 → 3)-Gal residue at the terminus did not show appreciable activity (Fig. 1B).

The presence of a glycolipid antigen as a cell surface marker characteristic for a specific type of tumor is becoming apparent in experimental cancer as well as in human malignancy. Ganglio-*N*-triaosylceramide (Gg3; asialo-GM_2) has been demonstrated to be a tumor-associated marker in mouse sarcoma (*16*) and lymphoma (*17*) and can be used as an effective target for suppression of tumor growth in vivo by passive immunization with a monoclonal antibody (immunoglobulin G3 class) directed to this glycolipid antigen (*18*). The use of monoclonal antibodies to analyze tumor-associated antigens has resulted in the discovery of a number of glycolipid antigens. A monosialoganglioside defined by an antibody specific to a human colonic tumor (*19, 20*) is a remarkable example. The structure of the antigen was recently identified as sialosyl-Le^a (*21*). A human melanoma antigen was defined by two monoclonal antibodies in two independent laboratories (*22*) and identified as GD3 ganglioside (*23*), with the ceramide having a long-chain fatty acid (*24*).

Globotriaosylceramide (P^k antigen), which has the unusual terminus structure Galα(1 → 4)-Gal (*9*), accumulates in various tissues and organs of patients with



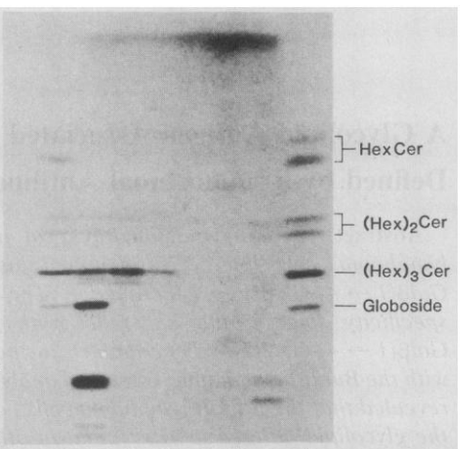


Fig. 2 (left). Lysis of liposomes containing the Burkitt lymphoma antigen, ceramide trihexoside, and various other glycolipids with complement and monoclonal antibody 38-13. The assay was performed by the method of Six *et al.* (*8*). Liposomes contained 10 μg of glycolipid, 500 nmole of dimyristoylphosphatidylcholine, 500 nmole of cholesterol, and 45 nmole of dicetylphosphate containing umbelliferone phosphate (*UP*). Lysis was measured by the release of umbelliferone phosphate (*8*). (●) Lysis of liposomes containing a purified ceramide trihexoside isolated from Burkitt lymphoma; the curve was obtained from mean values of two determinations. (○) No lysis of liposomes by various glycolipids, including globoisotriaosylceramide (*14*), cytolipin R (*35, 36*), and a ceramide pentasaccharide of rabbit erythrocytes (*15*). These glycolipids have the common structure α-Gal at the terminus. Globoisotetraosylceramide (cytolipin R; iGb4) (*35, 36*) prepared from rat kidney was donated by the late J. Kawanami; globoisotriaosylceramide (iGb3) (*14*) was prepared from globoisotetraosylceramide by treatment with jack bean β-*N*-acetylhexosaminidase (Sigma). Fig. 3 (right). High-performance thin-layer chromatogram of the neutral glycolipid fraction of Burkitt lymphoma and other lymphoproliferative disorders. Reference glycolipids are ceramide monohexoside (*HexCer*), dihexoside (Hex_2Cer), trihexoside (Hex_3Cer), and globoside. Lanes 1 to 3 are glycolipids of Burkitt lymphoma cell lines. (Lane 1) Ramos (EBV-negative North American type); (lane 2) P3HR1 (EBV-positive African type); and (lane 3) Daudi (EBV-positive African type). Lane 4 is T cell leukemia cell line JM; lane 5 is erythroleukemia cell line K562 (*13*); and lane 6 is lymphoblastoid cell line Priess. Cells were cultured in RPMI 1640 (Gibco) supplemented with 10 percent heat-inactivated calf serum. Glycolipids used for references were prepared from human erythrocytes (*30*).

Fabry's disease (*4, 5, 25*). Although globotriaosylceramide is present in moderate quantity in normal human erythrocytes (1.6 to 2.0 μg per 100 mg of dried cell residue; 0.08 to 0.10 μmole per 50 ml) (*26*), the quantity is 1/100 that in Burkitt lymphoma cells. Normal erythrocytes were not reactive to antibody 38-13 (*2*) but were weakly reactive to polyclonal antibodies to P absorbed by globoside (*5*). Analyses of the distribution, chemical quantity, and cell surface expression of this glycolipid in cells of other tissues are lacking. It is possible that expression of this glycolipid is cryptic, as it is in normal human erythrocytes and other blood cells. However, a certain strain of *Escherichia coli* causing urinary tract infections had a pili lectin that is known to interact with globoside and globotriaosylceramide (*27*). Whether the globotriaosylceramide of the genitourinary tract epithelia is exposed at the cell surface has not been determined.

The glycolipid associated with Burkitt lymphoma cell lines did not accumulate in other lymphoproliferative or myeloproliferative processes. Because this glycolipid accumulates only in Burkitt lymphoma cells, it could be used for the detection of Burkitt lymphoma cells among various malignant blood cells and lymphoid, erythroid, and myeloid leukemia cells. In a patient with gastric cancer, a rare genotype *pp* was associated with a P-like and P_1-glycolipid antigen. Since the tumor growth in that instance was suppressed by anti-P_1PP^k antibodies (*28*), the effect of monoclonal antibody 38-13 on Burkitt lymphoma growth is of great interest. Globotriaosylceramide accumulation in Burkitt lymphoma could result from a blockage of globoside synthesis or from activation of α-galactosyltransferase. Either mechanism could yield an altered profile in Burkitt lymphoma, analogous to changes generally associated with oncogenic transformation (*29*).

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Damselfish as Keystone Species in Reverse: Intermediate Disturbance and Diversity of Reef Algae

Abstract. *Substrates located within the defended territories of Hawaiian damselfish for 1 year were subjected to intermediate grazing intensity and, as a result, showed greater diversity of algae than substrates either protected within fish-exclusion cages or exposed to intense fish grazing outside territories. Thus, this damselfish enhances local diversity on reefs through "intermediate-disturbance" effects, and is a keystone species that decreases rather than increases overall predation intensity relative to areas where it is absent.*

A major goal of community ecology is to determine what factors allow similar species to coexist in the same habitat without one dominant competitively eliminating most others. One such mechanism is known as the "keystone-species" concept. As described by Paine (*1*), keystone species are predators that keep the population densities of their prey below levels where resources become limiting, thus preventing local competitive exclusions among the prey species. Paine (*1*) found that the controlled removal of a starfish population resulted in mussels excluding most other large invertebrates from a rocky intertidal zone. Thus, a keystone species can maintain high local diversity by increasing the intensity of predation relative to areas where that species is absent. We now describe a fundamentally different kind of keystone species: a damselfish that maintains high diversity of algae on Hawaiian coral reefs by reducing the overall intensity of predation on the algae (*2*). This system and the classic keystone-species concept are reconcilable when examined in the context of what has been called the "intermediate-disturbance" hypothesis (*3*), which predicts that local diversity reaches a peak at moderate levels of disturbance.

Herbivorous fishes affect the local distribution and abundance of algae on tropical reefs (*4–7*). Typically, intense grazing by aggregations of parrotfishes (Scaridae) and surgeonfishes (Acanthuridae) results in most exposed coral rock sur-