presence of a moving contour and its direction (*16*). The scheme is ideal in that it cannot be fooled by the emergence of new contours, inappropriate speed, or complicated texture (*17*). It represents a means of maintaining direction selectivity over a broad range of spatial and temporal frequencies to which cortical neurons are responsive, but is achieved by sacrificing sensitivity to image speed.

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2. Contrast (m), defined ($L_{max} - L_{min}/2L_0$, was 0.25; L_0 = 1.1 footlamberts (1 footlambert, 3.43 cd/m^2).
3. Horizontal gratings in vertical motion do not introduce horizontal disparity cues for depth perception and are less likely to stimulate vergence eye movements.
4. At higher contrasts, perceived motion is accompanied by flicker.
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7. The percept is degraded when phase differences other than 90° are used.
8. More complicated temporal modulation functions can be used provided they too exhibit quadrature relationships between left and right eye images.
9. Viewed binocularly, the patterns in Fig. 2 seem to change cyclically: the left pattern fades into the right, then to the negative (reversed contrast) image of the left, to the negative of the right, and back to the left. When the patterns are combined on a single display, the illusion resembles reversed phi [S. M. Anstis and B. J. Rogers, *Vision Res.* **15**, 957 (1975)].
10. On a test similar to the one preceding, each of three observers identified the direction of motion consistent with stimulus spatio-temporal frequency components (percent correct ≥85 for each pattern; $z \geq 3.13$; $P < 0.01$; $n = 20$ trials).
11. In practice this must be done by presenting the spatio-temporal line pattern for brief periods (for example, 250 to 500 msec), because ultimately the real opposing motion will produce a noticeable displacement. (At 114 cm the line contains spatial frequencies ≤25.6 cycle/deg).
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16. By "direction" we mean orthogonal to a neuron's preferred orientation ±90°.
17. The mechanism does not alias spatially or temporally.
18. We have adapted expressions derived by D. H. Kelly [*J. Opt. Soc. Am.* **69**, 1340 (1979)] describing human contrast sensitivity. The relation between contrast sensitivity data and the visual system's modulation transfer function is unknown and therefore serves only as an approximation.

19. We thank I. Ohzawa and R. D. Freeman for helpful comments on the manuscript and J. Slobin for volunteering her time. Supported by NIH grants EY01175 and EY05636 awarded to T.C. and R. D. Freeman.

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Receptor-Coupled Activation of Phosphoinositide-Specific Phospholipase C by an N Protein

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Cleavage of phosphatidylinositol 4,5-bisphosphate by phospholipase C results in the production of two important second messengers: inositol-1,4,5-trisphosphate and 1,2-diacylglycerol. Although several receptors promote this cleavage, the molecular details of phospholipase C activation have remained unresolved. In this study, occupancy of a Ca^{2+}-mobilizing receptor, the oligopeptide chemoattractant receptor on human polymorphonuclear leukocyte plasma membranes, was found to lead to the activation of a guanine nucleotide regulatory (N) protein by guanosine 5′-triphosphate. The activated N protein then stimulated a polyphosphoinositide-specific phospholipase C by reducing the Ca^{2+} requirement for expression of this activity from superphysiological to normal intracellular concentrations. Therefore, the N protein–mediated activation of phospholipase C may be a key step in the pathway of cellular activation by chemoattractants and certain other hormones.

THE MECHANISMS BY WHICH OCCUPANCY of certain hormone receptors leads to cellular activation by elevating intracellular Ca^{2+} levels have been the subject of intense investigation (*1, 2*). According to the current paradigm, agonist binding to these receptors leads to the phosphodiesteric cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2), yielding inositol-1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol. These products mediate the release of Ca^{2+} from intracellular stores (*2*) and the activation of protein kinase C (*3*), respectively. Studies of polyphosphoinositide-specific phospholipase C, however, have demonstrated that expression of this activity in vitro requires superphysiological concentrations of Ca^{2+} (>100 μ*M*) (*4–6*). Therefore, some other mechanism must exist for activating this enzyme at physiological Ca^{2+} concentrations (0.1 to 0.2 μ*M*).

Accumulating evidence suggests that a guanine nucleotide regulatory (N) protein is important in regulating the activity of phospholipase C. For example, addition of analogs of guanosine 5′-triphosphate (GTP) to permeabilized cells produces responses similar to those elicited by some hormonal stimuli (*7, 8*). In addition, *Bordetella pertussis* toxin, which catalytically inactivates certain N proteins (*9*), interferes with chemoattractant-induced responses of phagocytes including stimulated polyphosphoinositide hydrolysis (*10, 11*). More directly, we have recently shown (*12*) that occupancy of the oligopeptide chemoattractant receptor on human polymorphonuclear leukocyte (PMN) plasma membranes (*13*) by the chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe) leads to the hydrolysis of PIP_2 only if GTP is included during the incubation. Similarly, hydrolysis of PIP_2 in membranes prepared from blowfly salivary glands can be induced by the addition of serotonin and GTP (*14*).

In the present study, we defined the role of the N protein in the transduction mechanism of the oligopeptide chemoattractant receptor on human PMN's. When activated by a guanosine triphosphate, the N protein appears to reduce the concentration of Ca^{2+} required for activation of a polyphosphoinositide-specific phospholipase C to physiological levels.

Incubation of plasma membranes isolated from human PMN's with adenosine [γ-^{32}P]-triphosphate ([γ-^{32}P]ATP) leads to the synthesis of radiolabeled phosphatidic acid, phosphatidylinositol 4-phosphate (PIP), and PIP_2 (*12*). Further incubation of these labeled membranes with fMet-Leu-Phe (0.1 μ*M*) plus GTP (10 μ*M*), but not with either agent alone, in the presence of 1 μ*M* $CaCl_2$ resulted in hydrolysis of the labeled PIP_2 (Table 1). Guanosine di- and monophosphate (GDP and GMP), as well as ATP and adenosine di- and monophosphate (ADP and AMP), were not active in promoting PIP_2 breakdown either alone or in the presence of fMet-Leu-Phe. The GTP analog guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) stimulated PIP_2 hydrolysis both in the absence and presence of fMet-Leu-Phe. These results indicate that coupling of the occupied oligopeptide chemoattractant re-

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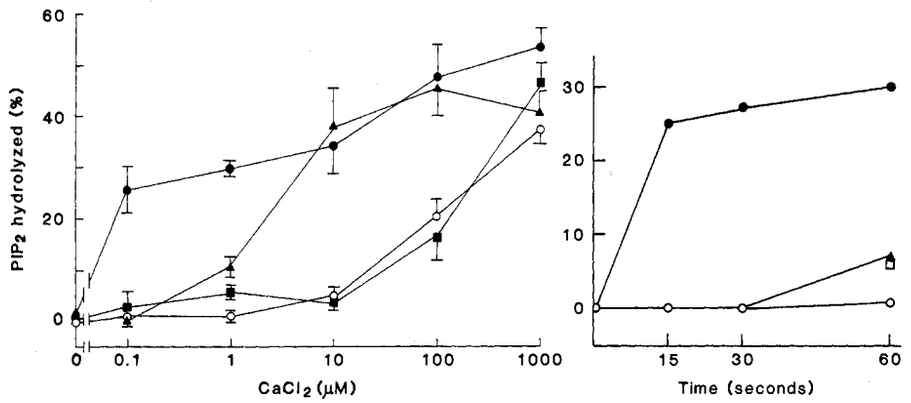

Fig. 1 (left). Ca^{2+}-dependence of PIP_2 hydrolysis in PMN plasma membranes. Plasma membranes (*33*) were phosphorylated as described in Table 1. The labeled membranes were then incubated for 60 seconds with the indicated concentrations of $CaCl_2$ in the presence of buffer alone (○), 0.1 μ*M* fMet-Leu-Phe (■), 10 μ*M* GTP (▲), or 0.1 μ*M* fMet-Leu-Phe plus 10 μ*M* GTP (●). Samples were then extracted and analyzed for [^{32}P]PIP_2 as described in Table 1. Values represent the mean percentage ± SEM of [^{32}P]PIP_2 hydrolyzed in 4 to 24 experiments. Fig. 2 (right). Time course of PIP_2 hydrolysis in PMN plasma membranes. Plasma membranes (*33*) were phosphorylated as described in Table 1. The labeled membranes were then incubated for the times indicated in the presence of 1 μ*M* $CaCl_2$ with no addition (○), 0.1 μ*M* fMet-Leu-Phe (■), 10 μ*M* GTP (▲), or 0.1 μ*M* fMet-Leu-Phe plus 10 μ*M* GTP (●). Samples were then extracted and analyzed for [^{32}P]PIP_2 as described in Table 1. Values represent the mean percentage of [^{32}P]PIP_2 hydrolyzed in two experiments that varied less than 5 percent.

ceptor with PIP_2 hydrolysis specifically requires a guanosine triphosphate.

Similar specificities have been demonstrated for several receptor systems that modulate adenylate cyclase activity through N protein intermediates (*15–17*). Inactivation of an N protein occurs by the hydrolysis of the bound GTP via its inherent guanosine triphosphatase activity (*18, 19*). Therefore, the GTP analogs 5′-guanylylimidodiphosphate (GppNHp) and GTPγS promote prolonged activation of N proteins because their terminal phosphorous groups are more resistant to hydrolysis. This effect is manifested by the ability of GppNHp and GTPγS to activate adenylate cyclase in the absence of a stimulatory agonist (*20*). Similarly, in the present study, PIP_2 hydrolysis was stimulated by GTPγS in the absence of fMet-Leu-Phe. Further evidence that the activation of PIP_2 hydrolysis in PMN plasma membranes is mediated by an N protein was provided by means of plasma membranes prepared from PMN's that had been treated with pertussis toxin; this treatment blocks responses of these cells to fMet-Leu-Phe (*10, 21–23*). In contrast to control plasma membranes, the pertussis toxin–treated membranes did not promote PIP_2 hydrolysis in the presence of fMet-Leu-Phe plus GTP at low Ca^{2+} concentrations (*12*). Therefore, the available evidence indicates that a pertussis toxin–inhibitable N protein is involved in the receptor-stimulated hydrolysis of PIP_2.

Plasma membranes isolated from PMN's contain a phospholipase C activity that can hydrolyze PIP_2 and PIP in the presence of high concentrations of Ca^{2+} (*5, 12*). The EC_{50} (that is, half-maximal hydrolysis of PIP_2) for this Ca^{2+}-induced hydrolysis of PIP_2 in PMN membranes was at approximately 200 μ*M* Ca^{2+} (Fig. 1). Addition of 0.1 μ*M* fMet-Leu-Phe did not significantly alter this Ca^{2+}-dependence for PIP_2 breakdown. However, addition of 10 μ*M* GTP (Fig. 1) or 100 μ*M* GTP reduced the Ca^{2+} requirement for PIP_2 hydrolysis by a factor of about 50 (EC_{50} ~4 μ*M* Ca^{2+}). Most important, addition of a combination of fMet-Leu-Phe and GTP (10 μ*M*) further reduced the Ca^{2+} dependence so that PIP_2 hydrolysis occurred with as low as 0.1 μ*M* Ca^{2+} (25.5 ± 4.5 percent of the PIP_2 hydrolyzed in 60 seconds). Therefore, it appears that the role of the GTP-activated N protein is to interact with a phospholipase, thereby allowing its activation at physiological concentrations of Ca^{2+}. The mechanism for this activation remains to be established, but it may involve potentiation of the affinity of the phospholipase for Ca^{2+} similar to that induced in protein kinase C by diacylglycerol (*3*).

Induction of PIP_2 hydrolysis by fMet-Leu-Phe plus GTP occurred rapidly, being nearly maximum at 15 seconds (Fig. 2). Therefore, this membrane system mimics the whole cell with regard to the speed and extent of receptor-induced PIP_2 hydrolysis. For example, exposure of intact PMN's to fMet-Leu-Phe leads to the hydrolysis of approximately 20 percent of the radiolabeled PIP_2 after 15 seconds, with no further loss of PIP_2 in the next 105 seconds (*10, 24*). Concurrent with this loss of PIP_2 in intact PMN's is a rapid production of IP_3 that disappears upon further incubation (*25*). Therefore, PIP_2 breakdown in intact PMN's is rapidly terminated, allowing for the resynthesis of PIP_2 and removal of IP_3. The mechanism for the termination of PIP_2 breakdown is unknown, but it could involve phosphorylation reactions mediated by an adenosine 3′,5′-monophosphate (cyclic AMP)–dependent protein kinase (*25*) or protein kinase C (*26, 27*).

Labeling of the polyphosphoinositides with [γ-^{32}P]ATP in vitro precluded the possibility of identifying the mechanism of the

Table 1. Nucleotide specificity for PIP_2 hydrolysis in PMN plasma membranes. Plasma membranes (approximately 25 μg of protein) isolated from human PMN's (*33*) were incubated for 60 seconds at 37°C with 100 μ*M* [γ-^{32}P]ATP (~1000 count/min per picomole) in the presence of 5 m*M* $MgCl_2$, 0.5 m*M* spermine, and 50 m*M* Hepes buffer (*p*H 7.0). An equal volume (50 μl) of 2 μ*M* $CaCl_2$ in 50 m*M* Hepes buffer (*p*H 7.0) containing 20 μ*M* of the indicated nucleotide and 0 or 0.2 μ*M* fMet-Leu-Phe was then added, and incubations were continued for 60 seconds. The reactions were terminated by the addition of 2 ml of chloroform:methanol (1:1 by volume), and phospholipids were extracted and analyzed by affinity chromatography (*34*) to determine the amount of radioactivity associated with PIP_2 (typically 500 to 800 count/min per 25 μg of protein in samples incubated with buffer alone). Values represent the mean percentage ± SEM (n = 3 or 4) of [^{32}P]PIP_2 compared with samples incubated with buffer alone.

Nucleotide added	[^{32}P]PIP_2 remaining (percent of control)	
	Without fMet-Leu-Phe	With 0.1 μ*M* fMet-Leu-Phe
None (buffer alone)	100	97 ± 9
GTP	99 ± 3	74 ± 5*
GDP	98 ± 3	99 ± 9
GMP	106 ± 9	93 ± 13
ATP	105 ± 10	97 ± 7
ADP	115 ± 12	100 ± 2
AMP	103 ± 4	103 ± 10
GTPγS	72 ± 5*	84 ± 4†

*P < 0.01 compared to buffer alone (paired t test). †P < 0.05 compared to buffer alone (paired t test).

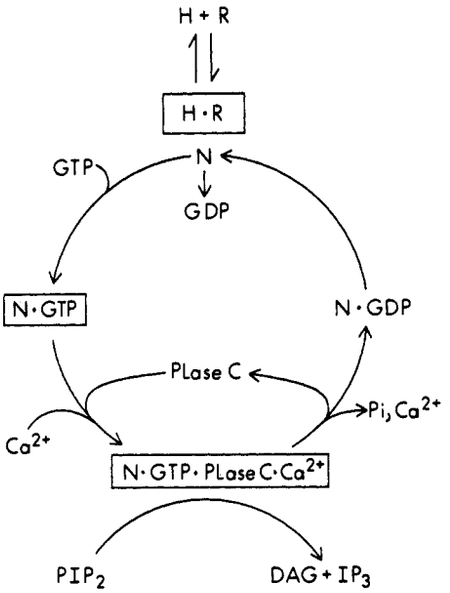

Fig. 3. Model for receptor-mediated stimulation of phospholipase C by an activated N protein complex. Boxed elements represent the activated forms of the indicated components. Binding of a Ca^{2+}-mobilizing hormone (H) or chemoattractant to its receptor (R) produces an activated hormone-receptor complex (H · R). This complex induces the exchange of a bound GDP with GTP on an N protein to yield an activated N protein species (N · GTP). In adenylate cyclase–coupled systems (*16, 17*), this activation process involves dissociation of the N protein heterotrimer, with the α subunit · GTP moiety being the activating unit. Similar reorganizations may occur in the system described here. The activated N protein may interact with a polyphosphoinositide-specific phospholipase C (PLase C), enabling it to express activity at physiological Ca^{2+} concentrations. The activated phospholipase cleaves PIP_2 into 1,2-diacylglycerol (DAG) and IP_3. The N · (or N_α · GTP · PLase C · Ca^{2+} complex is inactivated by the hydrolysis of the bound GTP, producing the inactive N · (or N_α · GDP. The N_α · GDP would then associate with the β and γ subunits, reforming the intact N protein. P_i, inorganic phosphate.

N protein–activated hydrolytic activity because IP_3, a presumed product of PIP_2 hydrolysis, cochromatographed with [γ-^{32}P]ATP. We therefore labeled the inositol phospholipids in human PMN's by incubating the cells for 24 hours with myo-[2-^{3}H]inositol in Medium 199 (Hazelton) containing 2.5 percent fetal calf serum. Under these conditions, the cells incorporated approximately 2 percent of the [^{3}H]inositol and synthesized ^{3}H-labeled phosphatidylinositol (PI), PIP, and PIP_2 containing 97, 2, and 1 percent of the incorporated [^{3}H]inositol, respectively.

Plasma membranes were isolated from these [^{3}H]inositol-labeled cells and treated with buffer, fMet-Leu-Phe, GTP, and Ca^{2+} (Table 2). The water-soluble ^{3}H-labeled products were then analyzed by ion exchange chromatography (*28*) and then by liquid scintillation spectrometry. Incubation of the labeled membranes with high concentrations of Ca^{2+} (1 m*M*) resulted in the production of significant amounts of ^{3}H-labeled inositol phosphates (Table 2), demonstrating the presence of a Ca^{2+}-activatable phospholipase C as described (*5*). These were exclusively [^{3}H]IP_2 and [^{3}H]IP_3, indicating that PIP and PIP_2, but not PI, are substrates for this phospholipase C. This specificity was further substantiated by the loss of [^{3}H]PIP and [^{3}H]PIP_2, but not [^{3}H]PI, upon incubation of the ^{3}H-labeled plasma membranes with 1 m*M* $CaCl_2$. Incubation of the ^{3}H-labeled membranes with GTP alone (50 μ*M*) caused the release of ^{3}H-labeled inositol phosphates (31 ± 12 percent of the amount released by 1 m*M* $CaCl_2$); however, the simultaneous addition of fMet-Leu-Phe (0.1 μ*M*) released even greater amounts (75 ± 4 percent of the high Ca^{2+} activity). Since the production of inositol phosphates can occur only through the action of a phospholipase C, the previously described losses of PIP_2 induced by activation of the N protein must be mediated, at least in part, by a phospholipase C.

Table 2. Production of inositol phosphates by N-activated phospholipase. Plasma membranes were isolated from human PMN's that had been incubated for 24 hours in Medium 199 containing 2.5 percent fetal calf serum (10^9 cells in 50 ml) and 1 mCi of myo-[2-^{3}H]inositol. The ^{3}H-labeled plasma membranes were then incubated for 60 seconds at 37°C with 50 m*M* Hepes buffer (*p*H 7.0) containing 1 m*M* $MgCl_2$ and 1 μ*M* $CaCl_2$ alone or with additions as indicated. The reactions were terminated by the addition of 2 ml of chloroform: methanol (1:1 by volume), and inositol phosphates were analyzed (*35*). For each experiment, the amount of ^{3}H present in the incubation with buffer alone (average, 1516 count/min) was subtracted as a blank, and the amount released by incubation with 1 m*M* $CaCl_2$ (average, 301 count/min more than blank) was taken as 100. The amount of ^{3}H-labeled inositol phosphates produced relative to this was calculated for each of the remaining experimental conditions. Values represent the mean ± SEM (n = 3) amount of ^{3}H-labeled inositol phosphates produced relative to that produced with 1 m*M* $CaCl_2$. The coefficient of variation averaged 4.3 percent for replicate samples.

Addition	^{3}H-labeled inositol phosphates produced
None	0
fMet-Leu-Phe (0.1 μ*M*)	9 ± 9
GTP (50 μ*M*)	31 ± 12
fMet-Leu-Phe (0.1 μ*M*) + GTP (50 μ*M*)	75 ± 4
$CaCl_2$ (1 m*M*)	100

Our present data, as well as our previous investigations, suggest a new mechanism for chemoattractant receptor–mediated activation of phagocytes.The oligopeptide chemoattractant receptor appears to be functionally coupled by a pertussis toxin–sensitive N protein to a phospholipase C that degrades PIP_2 to IP_3 and diacylglycerol (Fig. 3). This N protein may be N_i, since pertussis toxin catalyzes the ADP ribosylation of a 41,000 dalton protein in PMN membranes (*10, 21, 22*). We suggest that binding of the chemoattractant to its receptor leads to the activation of the N protein by GTP, possibly by lowering the affinity of N for GDP, thereby allowing nucleotide exchange. The activated N protein reduces the Ca^{2+} concentration required to activate a polyphosphoinositide-specific phospholipase C to resting intracellular levels. This mechanism may apply to a class of Ca^{2+}-mobilizing receptors, such as those for α_1-adrenergic agonists (*29*), muscarinic agonists (*30*), serotonin (*14*), and platelet activating factor (*31*), all of which appear to use N proteins. The involvement of N proteins in the regulation of at least three critically important second messenger systems [cyclic AMP (*16, 17*), cyclic GMP (*32*), and now IP_3 plus diacylglycerol] demonstrates the pivotal positions of this family of proteins in regulating cell function.

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33. PMN's (>95 percent purity) were isolated from blood obtained from healthy donors as described (*12*). Plasma membranes were prepared by N_2 cavitation and subsequent centrifugation for 60 minutes at 90,000*g* over a cushion of 41 percent sucrose [T. Maeda, K. Balakrishnan, S. Q. Mehdi, *Biochim. Biophys. Acta* **731**, 115 (1983)]. The membranes banding at the interface were recovered by centrifugation at 150,000*g* for 30 minutes, washed with 0.25*M* sucrose containing 10 m*M* Hepes buffer (*p*H 7.0), and then resuspended in the same buffer. Membranes were used immediately or frozen at −80°C. A single freeze-thaw cycle did not disrupt the coupling of the receptor and PIP_2 hydrolysis.
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Anti-Idiotypic Antibodies Bear the Internal Image of a Human Tumor Antigen

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Goat antibodies to idiotypes (anti-idiotypic antibodies; Ab2) that recognize an idiotype associated with the combining site of a BALB/c mouse IgG2a monoclonal antibody (Ab1) to human gastric carcinoma were used to immunize BALB/c mice and rabbits. A monoclonal anti–anti-idiotypic antibody (Ab3) of IgG1 isotype was obtained after immunization of mice. The Ab3 and the Ab1 showed identical binding specificities and bound with similar avidities to the same tumor antigen. The induction of Ab1-like Ab3 by Ab2 was not restricted to mice, since Ab3 could also be induced in rabbits. Both the mouse- and the rabbit-derived Ab3 bound the same gastrointestinal cancer-associated antigen as Ab1. These findings indicate that Ab2 induced the formation of antigen-specific Ab3, probably because it bears the internal image of the tumor-associated antigen. This Ab2 may therefore have potential for modulating the immune response of cancer patients to their tumors.

WE HAVE PREVIOUSLY DISCUSSED (*1, 2*) the possibility of a beneficial role of antibodies to idiotypes (anti-Id) induced in patients with gastrointestinal tract tumors after treatment with a monoclonal antibody (MAb). Direct evidence for a beneficial effect of anti-Id would be the triggering of an anti-tumor immune response in cancer patients after administration of anti-Id. Of the various populations of anti-Id directed against anti-tumor antibodies, those bearing the internal image of the tumor antigen will selectively stimulate antigen-specific B and/or T cells and will induce immunity in various species. Induction of antigen-specific immunity by anti-Id that most likely bear the internal image of the antigen has been reported in the rabbit allotype (*3*) and tobacco mosaic virus (*4*) systems. Immunization with anti-Id may play an important role in those cases where the antigen is not available. Since cancer antigens are not available in quantities sufficient for immunization, we have investigated the possibility that an internal image of the cancer antigen carried by an immunoglobulin (anti-idiotype) may produce an immune response against the tumor.

MAb GA733 (referred to hereafter as Ab1), with binding specificity for human carcinomas of various tissue origins, was chosen for production of anti-Id because of its tumoricidal activity in vitro and in vivo in nude mice (*5*). Tumor-associated antigen was purified from tumor cell lysates on an Ab1-linked affinity column (*6*). Anti-Id (Ab2) was produced in goats and isolated from the sera as described (*7*). The anti-idiotypic nature of affinity-purified goat Ab2 has been described previously (*2, 7*). The Ab2, at a concentration of 0.2 μg/ml, inhibited binding of iodinated Ab1 to target cells by 97 percent, indicating that Ab2 is directed to one or more epitopes within or near the combining site of Ab1. In control experiments, Ab2 did not significantly inhibit binding of an unrelated immunoglobulin G (IgG2a) MAb to the same target cells. The Ab2 population with combining site–related specificity represented approximately 67 percent of the total Ab2 population as determined by inhibition of binding of Ab2 to Ab1 by GA733 tumor-associated antigen. In a control, we observed no significant inhibition of binding of Ab2 to Ab1 GA733 in the presence of human serum albumin.

These results suggested that a major pop-

Table 1. Characterization of Ab1 (MAb GA733) and Ab3 CE5. Ab1 was generated as described (*5*). To generate Ab3, BALB/c mice were immunized with 50 μg of Ab2 in complete Freund's adjuvant subcutaneously and were injected on day 11 with the same amount of Ab2 in incomplete Freund's adjuvant, and again intravenously without adjuvant on day 33. Three days later, spleen cells were fused with non-secretor 653 mouse myeloma cells (*8*). Antibody isotype was determined by Ouchterlony immunodiffusion. Binding of ^{125}I-labeled GA733 antigen to either Ab1 or Ab3 was tested in a solid-phase radioimmunoassay (*18*). Antibody avidity and number of antibody-binding sites per cell were determined by Scatchard analysis (*19*). ADMC against [^{3}H]thymidine-labeled CRC cells was determined as described, by means of thioglycollate-elicited murine peritoneal macrophages as effector cells (*9*). Inhibition of binding of ^{125}I-labeled Ab1 to Ab2 by Ab3 or Ab1 was measured by a solid-phase radioimmunoassay (*18*) in which Ab2 at a concentration of 0.5 μg/ml was bound to wells of polyvinyl microtiter plates and then the wells were incubated with various concentrations of Ab3 CE5 or Ab1 GA733. Finally, the wells were incubated with ^{125}I-labeled Ab1 (20,000 count/min per well) and inhibition of binding of ^{125}I-labeled Ab1 was calculated.

Parameter investigated	Ab1 (MAb GA733)	Ab3 CE5
Isotype	IgG2a	IgG1
Binding to ^{125}I-labeled GA733 antigen (count/min)	10,905.0	1,410.0
Binding avidity (association constant $K_a \times 10^7 M^{-1}$) ± SEM	2.0 ± 1.0	4.0 ± 1.0
Number of Ab-binding sites per cell ($\times 10^6$) ± SEM	3.0 ± 0.5	2.7 ± 0.1
ADMC (% specific lysis)	57.4	25.1
Maximum inhibition of binding of Ab1 to Ab2 (%)	95.6	41.0

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