Cellular Immunology **191,** 1–9 (1999)
Article ID cimm.1998.1401, available online at http://www.idealibrary.com on IDEAL®

Carbohydrate-Bearing Cell Surface Receptors Involved in Innate Immunity: Interleukin-12 Induction by Mitogenic and Nonmitogenic Lectins

Eric Muraille, Bernard Pajak, Jacques Urbain, and Oberdan Leo

Laboratoire de Physiologie Animale, Département de Biologie Moléculaire, Université Libre de Bruxelles, B-1640 Rhode-St-Genèse, Belgium

Received June 17, 1998; accepted September 29, 1998

Based on the observation that pathogen-derived lectins play an important role in cell adhesion and invasion, we examined the possible role of host carbohydrate-bearing molecules in inducing the secretion of IL-12, a crucial proinflammatory cytokine. The ability of 12 plant lectins to recognize and stimulate naive murine mononuclear cells *in vitro* has been characterized in this study. Mitogenic lectins (comprising Con A, PHA, PSA, and LCA) were found to induce the secretion of multiple cytokines *in vitro,* including IL-2, interferon (IFN)-γ, and IL-12. Of interest, WGA, a nonmitogenic lectin unable to promote IL-2 secretion, was found to induce IL-12 and IFN-γ production in a T and B cell-independent fashion. The functional properties of WGA were inhibited by *N*-acetylneuraminic acid and *N,N′*-diacetylchitobiose. WGA therefore represents a potentially useful tool for the study of membrane glycoproteins involved in the early proinflammatory response characteristic of innate immunity.

Key Words: **lectin; interferon-γ; interleukin-12.**

INTRODUCTION

Infection caused by numerous pathogenic agents is often initiated by their adherence to host cell surface components via specific pathogen proteins (thereby referred to as adhesins). Numerous adhesins specifically interact with carbohydrate-containing structures and are therefore classified as lectins (reviewed in Ref. 1). This form of direct pathogen–host recognition (independent of serum components, opsonins, such as IgG, collectins, or IC3b fragment of complement, for example) has recently attracted interest as it not only provides a means for several pathogens to gain access to selected host compartments, but may also represent a potential host defense mechanism. Indeed, the role of direct pathogen–host recognition in provoking local inflammation has been recently documented. It has been observed that following interaction with pathogens, immune effectors, such as neutrophils or macrophages, may release into the extracellular media proinflammatory agents (such as reactive oxygen intermediates and arachidonic acid) and cytokines (IL-1, IL-6, PAF, and TNF-α) (2–5). However, despite its important role in the pathophysiology associated with infection, our knowledge of this recognition process remains in its infancy.

IL-12 is a heterodimeric cytokine (p70, of molecular weight 70 kDa) composed of a 40-kDa subunit (p40) disulfide-linked to a 35-kDa subunit (p35) and produced by various cell population, such as macrophages/monocytes (6), dendritic cells (7), and transformed B cell lines (8). IL-12 stimulates both T (9) and NK cells (10) to produce interferon (IFN)-γ, which in turn activates macrophages, leading to enhanced clearance of the invading organism (11). Multiple studies have suggested that IL-12 production represents a key event in the early immune response to pathogens, as shown by the observation that microbial products known to mimic a natural infection (such as LPS, bacterial DNA, and synthetic double-stranded RNA) induce IL-12 secretion (12–14). Thus, the identification of host structure participating in pathogen recognition and IL-12 production may be of prime importance.

Although their basic biological function is not fully understood, plant lectins have provided a well-established, ligand-based approach for specifically identifying host-expressed carbohydrate moieties involved in pathogen recognition. Indeed, because of the specificity that each lectin has toward a particular carbohydrate structure, even oligosaccharides can be distinguished. To search for cell surface carbohydrate structures potentially involved in the early inflammatory response to pathogens, we examined the ability of 12 well-characterized plant lectins to induce lymphoid cell aggregation, proliferation, and proinflammatory cytokine production, in conjunction with the distribution of their

0008-8749/99 $30.00

glycoprotein receptor. This study has led to the identification of lectins able to induce IL-12 production.

MATERIALS AND METHODS

Mice and Reagents

Female BALB/c, CB17 control, and CB17 SCID 6- to 8-week-old mice were purchased from Charles River Wiga (Sulzfeld, Germany) and maintained in a pathogen-free environment in our own animal facility. Mice genetically deficient for the p40 chain of IL-12 (15) were backcrossed into the Balb/c background and were kindly provided by J. Magram (Hoffman-LaRoche Inc., Nutley, NJ). The following antibodies to murine determinants were used in this study: C17.8.20.15 (anti-p40 IL-12, rat IgG2a), kindly provided by Dr. G. Trinchieri (Wistar Institute, Philadelphia, PA); and a rat IgG2a anti-idiotypic antibody to a mouse IgM myeloma protein, BCL-1, kindly provided by Kris Thielemans (Vrije Universiteit Brussel, Brussel, Belgium). All plant lectins used in this study were purchased from Vector Laboratories (Burlingame, CA). *N*-Acetylneuraminic acid (NANA) and *N,N′*-diacetylchitobiose (GlcNAcβ1-4GlcNAc) were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Purification

Unselected spleen and thymus-derived cells were obtained following mechanic dissociation of freshly removed organs. Splenic dendritic cells were purified as previously described (16) and contained more than 95% of $CD11c^+$ cells and less than 1% $CD3^+$ cells. Peritoneal macrophages were purified as previously described (17). The resulting population contained at least 90% macrophages, as assessed by morphology and specific staining using Mac-1 mAb specific to CD11b (ATCC).

Flow Cytometry

Cells were analzyed by flow cytometry with a FACScan cytometer (Becton–Dickinson, Mountain View, CA). Cells were incubated with biotin-coupled lectins and stained by FITC-coupled avidin. When indicated, cells were preincubated with 2.4G2 (a rat anti-mouse Fc receptor mAb) for 10 min prior to staining to prevent antibody binding to FcR and further labeled with phycoerythrin-coupled antibodies to murine CD45R and CD90 (Pharmingen, San Diego, CA). Cells were gated according to size and scatter in order to eliminate dead cells and debris from analysis. The blastogenesis index was calculated as the ratio of mean channel fluorescence (forward light scatter, arbitrary units on a linear scale) of lectins stimulated versus a control cell population.

In Vitro Responses

All *in vitro* immune responses were performed in serum-free medium containing RPMI 1640 (Seromed; Biochem KG, Berlin, Germany) supplemented with 2% HY ultroser (Gibco BRL), penicillin, streptomycin, nonessential aminoacids, sodium pyruvate, 2-ME, and L-glutamine (Flow ICN Biomedicals, Bucks, UK). Spleen cells or thymocytes (4×10^5 cells/well) were stimulated by lectins in a total volume of 0.2 ml in 96-well U-bottom plates. Cultures were maintained at 37°C in a humidified incubator (7% CO_2). Proliferation was assessed by [^{3}H]thymidine incorporation. The proliferation index represents the ratio of cpm of stimulated and control cultures.

Aggregation Assays

Cell populations were washed and plated at 5×10^5/well in 96-well flat-bottom plates. Cell aggregation was scored by two independent observers on a semiquantitative scale ranging from 0 to 5+ according to Rothlein *et al.* (18): 0, no aggregation; 1+, less than 10% of the cells in aggregates; 2+, 10–50% of the cells in aggregates, 3+, 50–100% of the cells in small, loose clusters; 4+, up to 100% of cells aggregated in large clusters; and 5+, nearly 100% of cells in large, very compact aggregates.

Cytokine Determination

For the IL-2 assay supernatants were collected after 24 h of culture, frozen, and assayed for IL-2 content by a bioassay using a subclone of the CTL.L cell line insensitive to murine IL-4 (19). Proliferation was assessed by [^{3}H]thymidine incorporation. Standard curves were generated using human rIL-2 and results are expressed as U/ml. IFN-γ and p40 IL-12 levels were determined by two-site ELISA using anti-IFN-γ mAb F1 and Db-1 (kindly provided by Dr. Billiau, K.U.L., Leuven, Belgium; and P. H. Van Der Meide, TNO Health Research, Rijswijk, The Nederlands, respectively) and anti-p40 mAb 5D9 and SC3-POD, both provided by David H. Presky (20). Standard curves were generated using purified recombinant mIFN-γ (kindly provided by Dr. Billiau) and supernatant containing mIL-12 (kindly provided by Dr. Thielemans). Results are expressed as U/ml.

RESULTS

Flow Cytometric Analysis of Splenic, Thymic, and Peritoneal Cell Populations Using a Panel of 12 Biotin-Coupled Lectins

The plant lectins used in this study are listed in Table 1, including their known sugar-binding specificity. The binding properties of these lectins on selected murine immune cell populations were examined by flow cytometry using biotin derivatives (Table 2). The four lectins Con A, JAC, LCA, and PSA bound to all

TABLE 1

Systematic name (common name)	Acronym	Sugar residues or sequences/ oligosaccharide structures binding preference[a]
Canavia ensiformis (jack bean)	Con A	α-Linked Man[b]
Dolichos biflorus (horse gram)	DBA	α-linked GalNAc
Datura stramonium (thorn apple)	DSL	Terminal or internal GlcNAcβ1-4GlcNAc
Griffonia simplicifolia	GSA-I	Terminal αGal and αGalNAc
Griffonia simplifolia	GSA-II	Terminal α,βGlcNAc
Artocarpus integrifolia (Jackfruit)	JAC	*O*-Glycosidically linked oligosaccharides Galβ1,3GalNAc
Lens culinaris (lentil)	LCA	Fucosylated core region of bi- and triantennary *N*-glycosically linked oligosaccharides containing α-linked Man
Phaseolus vulgaris erythroagglutinin (kidney bean)	PHA-E	Bisected bi- and triantennary complex N-linked sequences
Phaseolus vulgaris leukoagglutinin (kidney bean)	PHA-L	Highly branched (in triantennary or more) nonbisected complex sequences
Pisum sativum (garden pea)	PSA	α-Linked Man-containing oligosaccharides with an *N*-acetylchitobiose-linked αFuc residue included
Ulex europaeus (gorse seed)	UEA-1	α-Linked Fuc residues
Triticum vulgare (wheat germ)	WGA	NeuAc and oligosaccharides containing terminal GlcNAc or GlcNAcβ1-4GlucNAC (chitobiose)
	sWGA	Does not bind sialic acid resisues

[a] As described in (21).
[b] Man, mannose; Glc, glucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Fuc, fucose; NeuAc, neuraminic acid.

splenic cell populations tested, including, in particular, T cells. GSA-1, DSL, PHA-E, and PHA-L displayed a preferential binding to APC populations (B and/or dendritic cells) compared to T cells, while WGA selectively bound to non-T cells. The DBA lectin reacted weakly with the total splenic population but was nevertheless found to stain purified splenic dendritic cells above background levels. Finally, two lectins (UEA-1 and GSA-II) were found to be negative on all cell populations tested and will be used as negative controls throughout this study.

In Vitro Functional Properties of Plant Lectins on Murine Leukocytes

The functional characteristics of the lectins used in this study are shown in Table 3. Their mitogenic properties, ability to induce cell size increase, and cell aggregation were determined on both spleen cells and thymocytes as described under Materials and Methods. Cytokine production was only studied on spleen cell populations, according to standard procedures. The six lectins GSA-I, GSA-II, UEA-1, DBA, DSI, and JAC did not activate splenic cells to cytokine secretion. In contrast, the lectins Con A, LCA, PHA-E, PSA, and to a lesser extent, PHA-L were found to induce cell proliferation and, for most lectins, both IL-2 and IFN-γ secretion. These lectins were also able to induce thymocyte proliferation *in vitro* (see Table 3). In contrast to the aforementioned groups, the WGA lectin induced detectable levels of INF-γ production, despite its lack of mitogenic and IL-2-promoting properties. Note that in contrast to most mitogenic lectins, WGA induced cell aggregation but no cellular proliferation *in vitro*.

Induction of Proinflammatory Cytokine Production by Mitogenic and Nonmitogenic Lectins

As the original goal of this study was to identify lectins able to activate accessory cells to proinflammatory cytokine secretion, the *in vitro* IFN-γ response was further examined. Six lectins were found to induce detectable IFN-γ production *in vitro* from murine

TABLE 2

	SC[a]	B cells[b]	T cells[b]	DC[a]	Mac[a]	Thymocytes[a]
Unstained	1[c]	1	1	1	1	1
Con A	108	135	148	192	54	202
DBA	1	1	2	3	1	1
DSL	12	20	5	11	9	53
GSA-I	14[d]	28	6	11	3	33
GSA-II	1	1	1	1	1	1
JAC	9	4	11	6	3	11
LCA	238	256	261	448	214	223
PHA-E	85[d]	126	47	282	50	128
PHA-L	34[d]	48	15	14	24	60
PSA	85	80	119	124	74	77
UEA-I	1	1	1	1	1	1
WGA	18[d]	29	2	32	3	11

[a] Cell populations were isolated and purified as described under Materials and Methods.
[b] Determination of the binding activity of the biotin-labeled lectins on splenic subpopulations was performed by two-color analysis of spleen cells stained by PE-coupled antibodies to CD45R and CD90.
[c] These data are collected from a single experiment representative of three and are expressed as the ratio of mean channel fluorescence (FL1, log scale) of stained versus control, avidin–FITC-incubated cells.
[d] Staining on unselected spleen cells was heterogenous, with at least two distinct peaks of fluorescence.

TABLE 3

Responder populations:	Spleen cells								Thymocytes		
Assay[a]:	Aggregation (Score)	Blastogenesis (Index)	Proliferation (Index)	IL-2 (24 h) (U/ml)		IFN-γ (24 h) (U/ml)		P40 IL-12 (48 h) (U/ml)	Aggregation (Score)	Blastogenesis (Index)	Proliferation (Index)
Lectin doses (μg/ml):	1	1	1	3	1	3	1	1	1	1	1
Unstimulated	0	1.0	1	—[b]	—	—	—	—	0	1.0	1
Group 1[c]											
GSA-II	0	1.0	1	—	—	—	—	—	0	1.0	1
UEA-I	0	1.0	1	—	—	—	—	—	0	1.0	1
Group 2											
DBA	0	1.0	1	—	—	—	—	—	0	1.0	1
DSL	0	1.0	1	—	—	—	—	—	0	1.6	1
GSA-I	0	1.0	1	—	—	—	—	—	2	1.2	1
JAC	0	1.1	1	—	0.1	—	—	—	2	1.1	1
Group 3											
Con A	3	1.4	26	0.5	15.3	86	215	4	3	1.7	359
LCA	5	1.4	21	1.1	2.4	31	62	22	5	1.3	93
PHA-E	4	1.1	7	0.5	0.1	51	45	7	5	1.4	5
PHA-L	2	1.0	1	0.1	—	18	9	2	4	1.3	2
PSA	5	1.4	20	6.2	2.3	22	44	24	2	1.2	81
Group 4											
WGA	2	1.0	1	—	—	16	31	12	1	1.1	1

[a] All assays were performed as described under Materials and Methods and the results are representative of three independent determinations.
[b] Below the detection limits of the assay (<0.02 U/ml for IL-2, <1 U/ml for IFN-γ, and <0.2 arbitrary U/ml for p40 IL-12).
[c] See discussion for group determinations.

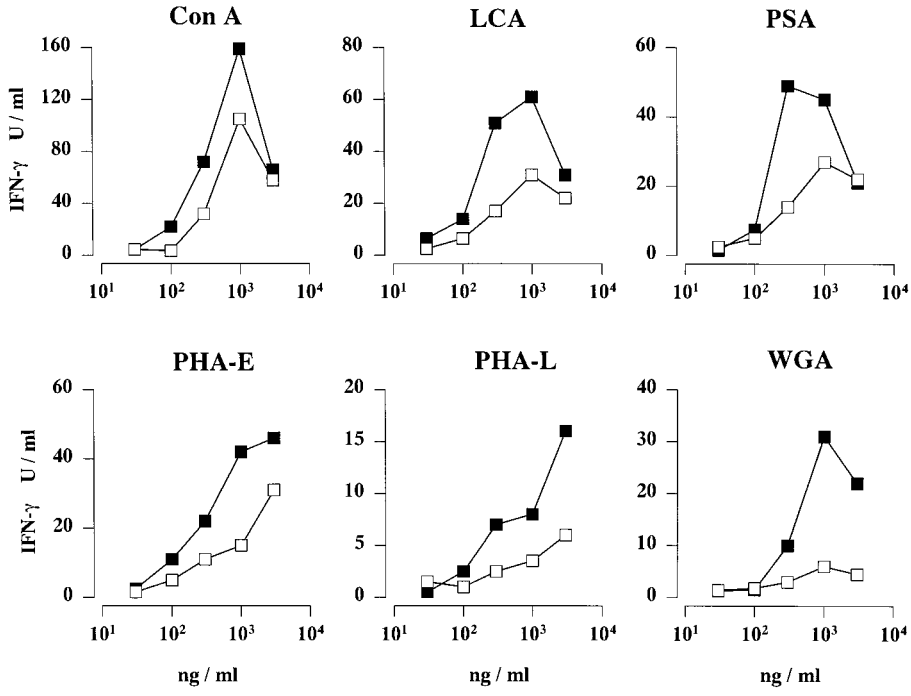

FIG. 1. IL-12-dependent IFN-γ production induced by lectins. Murine spleen cells (4×10^5 cells/0.2-ml culture) were stimulated *in vitro* by graded doses of lectins in the presence of 1% of culture supernatant of hybridoma lines producing control rat antibodies (■) or antibodies to murine p40 IL-12 (□), and IFN-γ production was determined following 24 h of culture by specific ELISA as described under Materials and Methods. The figure represents data collected from a single experiment representative of three.

spleen cells (Con A, LCA, PHA-E, PHA-L, PSA, and WGA, see Table 3). Supernatants of lectin-stimulated spleen cells were further assayed for p40 IL-12 production by ELISA (Table 3). Significant p40 IL-12 production was observed in cultures stimulated by all IFN-γ-inducing lectins. Note that p70 IL-12 heterodimer was not detected in the supernatant of lectin-stimulated cultures using a recently described (20) p70-specific ELISA (data not shown). To evaluate the potential role of endogenous IL-12 in the lectin-induced IFN-γ production, splenic murine cells were stimulated *in vitro* in the presence of saturating amounts of a blocking monoclonal antibody to murine IL-12. As shown in Fig. 1, antibodies to IL-12 inhibited lectin-induced IFN-γ secretion to a variable extent. In particular, while most lectins were able to induce significant IFN-γ production in the presence of anti-IL-12 antibodies, WGA-induced IFN-γ secretion appeared to be strictly dependent upon endogenous IL-12 production (Fig. 1). The IFN-γ production in response to the lectins Con A, LCA, PSA, and PHA was only partially inhibited by anti-IL-12 antibodies, suggesting that both IL-12-dependent and IL-12 independent pathways coexist for the induction of IFN-γ secretion in response to these lectins. To further confirm these findings, mice genetically deficient for the p40 chain were stimulated *in vitro* according to the same protocol. As shown in Fig. 2, p40 −/− mice were unable to produce IFN-γ in response to WGA stimulation, while secreting detectable levels of this cytokine in response to LCA, PSA, and PHA. Unexpectedly, disruption of the p40 gene strongly inhibited the ability of mice to secrete IFN-γ in response to Con A (Fig. 2), although Con A-induced IFN-γ secretion appeared to be relatively resistant to anti-IL-12-mediated inhibition (see Fig. 1). Although beyond the scope of this study, this observation may suggest that IL-12 KO mice lack a particular subpopulation of effector cells able to produce IFN-γ in response to Con A. This hypothesis is presently under investigation.

Carbohydrate and Cellular Requirement for WGA-Induced IL-12 Production

To insure that WGA-induced proinflammatory cytokine secretion was dependent upon interaction of the lectin with a carbohydrate-associated structure on immune cells, the ability of soluble sugar ligands to inhibit the *in vitro* functional properties of this lectin was assayed. Spleen cell suspensions were incubated with 20 μg/ml of DiGlcNAcβ1-4GlcNAc or NANA known to represent WGA ligands (21) before lectin stimulation. As shown in Fig. 3, both WGA-specific sugars strongly inhibited WGA-induced IFN-γ and p40 IL-12 production, while unaffecting the response to the unrelated lectin LCA (specific for α-D-glucose and α-D-mannose).

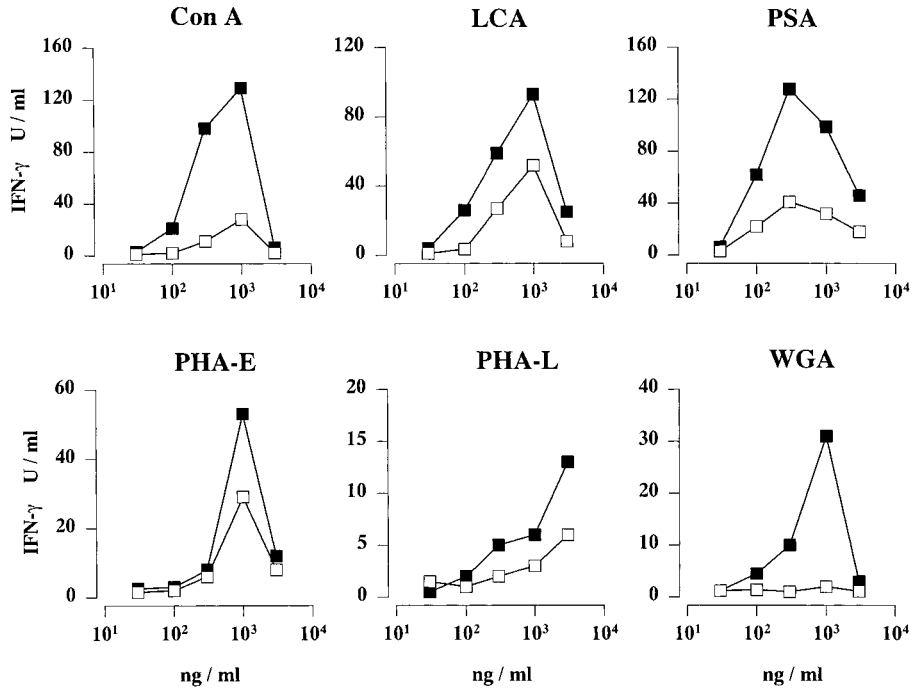

FIG. 2. Role of endogenous IL-12 in lectin-mediated IFN-γ production. Murine spleen cells (4×10^5 cells/0.2-ml culture) from +/+ mice (■) or p40 IL-12 −/− mice (□) were stimulated *in vitro* by graded doses of lectins, and IFN-γ production was determined following 48 h of culture by specific ELISA as described under Materials and Methods. The figure represents data collected from a single experiment representative of three.

Of note, succinylated WGA, which has unaltered affinity for *N*-acetylglucosamine but does not bind NANA residues (21), was inactive, suggesting an important role for NANA-containing glycoreceptors in mediating the functional properties of WGA. These observations exclude the role for contaminating endotoxins, and demonstrate that cell surface receptors expressing WGA-specific ligands can lead to cellular activation and secretion of inflammatory cytokines.

Finally, the cellular requirement for lectin-induced IL-12 production was assayed by using T and B cell-deficient mice as responders. Splenic cell populations from control and SCID mice were stimulated *in vitro* as previously described and the secretion of IL-2, IFN-γ, and p40 IL-12 was determined. As expected, SCID mice were found to respond weakly to the mitogenic lectins Con A, LCA, PHA-L, and PSA (Fig. 4). This observation is in agreement with the hypothesis that IL-12 production by these lectins is a consequence of polyclonal T cell stimulation. Activated T cells express CD40L molecules (22), and are able to induce both macrophages (23) and dendritic cells (24, 25) to IL-12 secretion in a CD40-CD40L-dependent fashion. In marked contrast, WGA-induced IFN-γ and IL-12 production was unaffected by the SCID mutation, suggesting that this lectin may exert its stimulatory properties by directly interacting with a non-T, non-B accessory cell population.

DISCUSSION

Based on the present observations and for the sake of discussion, the lectins characterized in this study have been tentatively classified into four groups. The first group includes lectins (namely, GSA-II and UEA-1), for which no significant binding nor functional activity could be demonstrated in our experimental setting. The second group is composed of lectins (DBA, GSA-I, DSL, and JAC) which failed to functionally affect murine spleen cells *in vitro* (note the failure to induce cell aggregation) despite detectable binding capacities (as assayed by flow cytometry). Lectins displaying a detectable to strong mitogenic activity and the ability to induce both IL-2 and IFN-γ secretion *in vitro* belong to a third group. This group includes widely studied lectins such as Con A and PHA, as well as the LCA and PSA lectins. Note that our observations revealed a strong correlation between a high binding capacity and the ability to functionally activate naive lymphocytes *in vitro* (compare Tables 2 and 3). Finally, the fourth group comprises one lectin (WGA), which despite its absence of mitogenic or blastogenic properties, induces the secretion of the proinflammatory cytokines IL-12 and IFN-γ (Table 3).

The observations reported herein strongly suggest that the WGA lectin is able to activate accessory-like cells to cytokine production. Indeed, this lectin prefer-

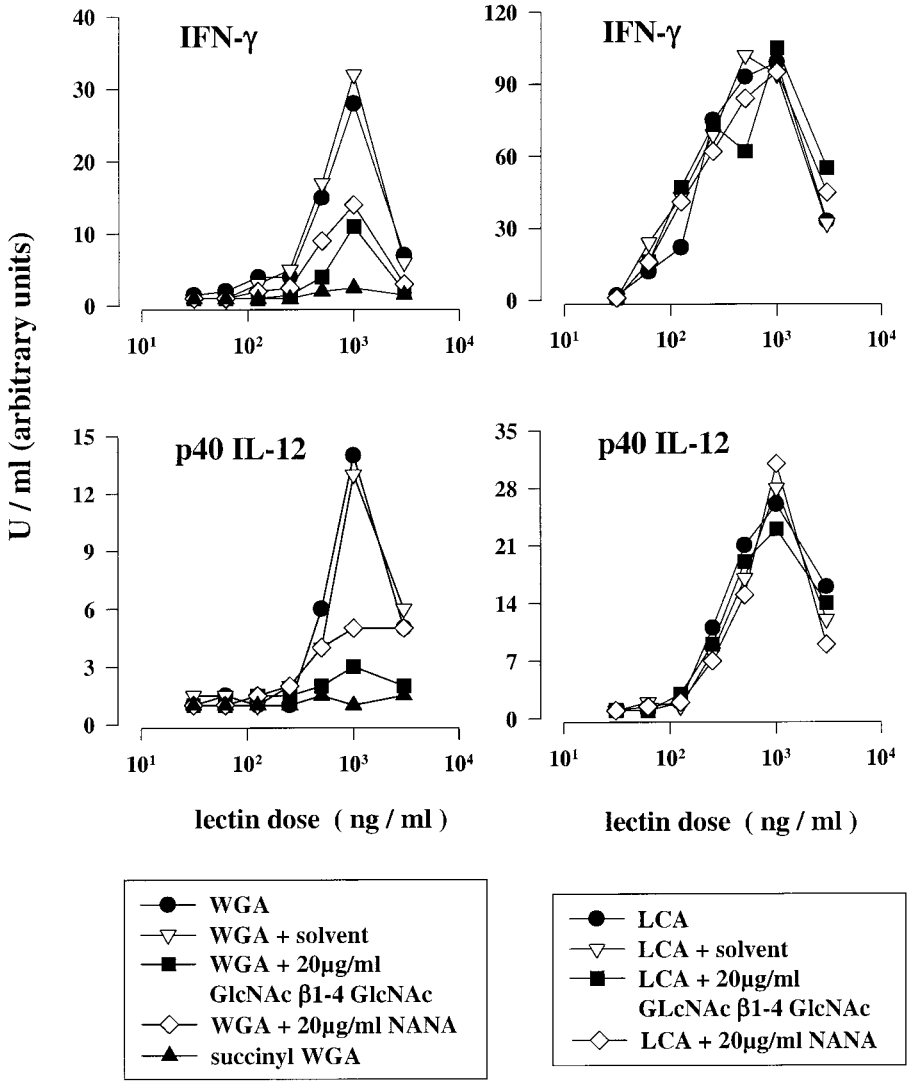

FIG. 3. Inhibition of WGA-induced proinflammatory cytokine production by sugars. Murine spleen cells (4×10^5 cells/0.2-ml culture) were stimulated *in vitro* by graded doses of lectins in the presence of *N*-acetylneuraminic acid (NANA) and *N,N′*-diacetylchitobiose (GlcNAcβ1-4GlcNAc), and IFN-γ and IL-12 production were determined following 48 h of culture by specific ELISA as described under Material and Methods. The figure represents data collected from a single experiment representative of three.

entially binds to the antigen-presenting cell population (including B lymphocytes and dendritic cells, see Table 2) and is able to activate spleen cells from SCID mice (lacking both T and B cells) to IL-12 production *in vitro* (Fig. 3). Although IL-12 was originally discovered as a soluble factor produced by EBV-transformed human B cell lines, normal B cells do not seem to produce significant amounts of this cytokines (26). In contrast, accessory cells (including monocytes, macrophages, and dendritic cells) seem to represent the major source of IL-12 *in vivo* (27). Thus, WGA appears to activate accessory cells to IL-12 production in a T and B cell-independent fashion (see Fig. 2), which in turn promotes IFN-γ secretion from T and/or NK cells. Note that our study is in agreement with a previous report (28), which shows that WGA is able to activate human leukocytes to IFN-γ production. In contrast, a study performed by Y. Ito *et al.* (29) on murine spleen cells revealed that WGA only induced IFN-α/β, but not IFN-γ production. The reasons for this discrepancy are not known but may be related to different lectin origins or to distinct experimental procedures used for IFN-γ detection (bioassay versus ELISA).

The *in vitro* functional properties of WGA were strongly inhibited by NANA and by a *N*-acetylglucosamine(GlucNAc)-containing sugar, in agreement with the known binding specificity of this lectin (see Table 1). Of particular interest, NANA (30) and GlcNac (31–34) are sugars associated to several mammalian cell surface glycoproteins known to represent likely receptors for several viruses (such as influenza A and B virus (30), human immunodeficiency virus (31), and

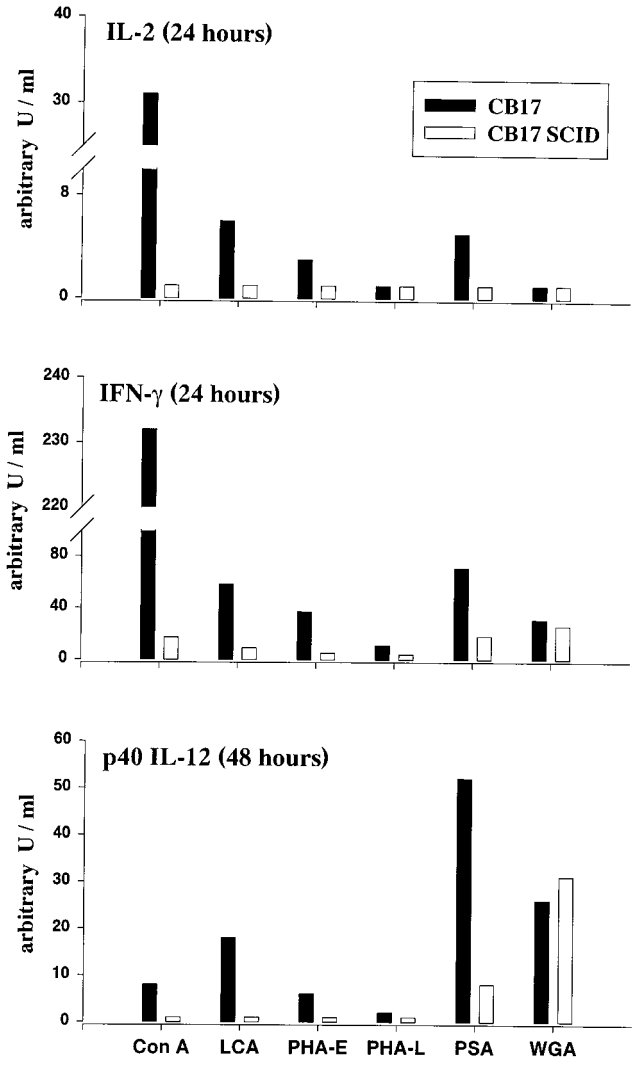

FIG. 4. Role of antigen–receptor-expressing lymphocytes in IL-12 production induced by lectins. Murine spleen cells (4×10^5 cells/0.2-ml culture) were stimulated *in vitro* by lectins (1.0 μg/ml), and p40 IL-12 production was determined following 48 h of culture by specific ELISA as described under Materials and Methods. The figure represents data collected from a single experiment representative of three.

cytomegalovirus (31, 32)), bacterial toxins (such as cholera toxin, tetanus toxin, and pertussis toxin (30)), or bacterial strains (such as *Pseudomonas aeruginosa* (33) and *Streptococcus pneumoniae* (34)), suggesting that glycoconjugates containing these sugars may play an important role in the recognition of microbial structures during infection.

The ability to interact with selected mammalian glycoproteins has been generally considered as a strategy developed by pathogens during evolution as an efficient mechanism for gaining access to the host intracellular milieu. Based on the present study, we suggest that mammalian hosts have taken advantage of these carbohydrate–lectin interactions by associating selected cell-surface glycoconjugates to activation of the innate immune response. Innate immunity not only provides a rapid host antimicrobial defense, but also plays an important role in determining how the adaptive, clonal, immune response reacts toward pathogen-derived antigens (35, 36). In particular, the cytokine IL-12 is known to promote the differentiation of $CD4^+$ helper cells toward a Th1-like phenotype, well suited for the elimination of most intracellular pathogens (37).

In conclusion, WGA may represent an useful tool for identifying cell surface glycoconjugate receptors and transduction signals involved in the production of inflammatory cytokines in response to infection.

ACKNOWLEDGMENTS

The authors thank G. Dewasme, M. Swanepoel, F. Tielemans, and P. Vaerman for expert technical assistance; H. Presky, K. Thielemans, and G. Trinchieri for providing reagents; and M. Moser for carefully revising the manuscript. This work presents research results of the Belgian Program in Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming.

REFERENCES

1. Ofek, I., Goldhar, J., and Keisari, Y., *Annu. Rev. Microbiol.* **49,** 239, 1995.
2. Konig, B., and Konig, W., *Infect. Immun.* **59,** 3801, 1991.
3. Rautelin, H., Blomberg, B., Järnerot, G., and Dannielsson, D., *Scand. J. Gastroenterol.* **29,** 128, 1994.
4. Venture, Y., Scheffer, J., Hacker, J., Goebel, W., and Koning, W., *Infect. Immun.* **58,** 1500, 1990.
5. Weiss, S. J., *N. Engl. J. Med.* **320,** 365, 1989.
6. Hsieh, C.-S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A., and Murphy, K. M., *Science* **260,** 547, 1993.
7. Macatonia, S. E., Hosken, N. A., Litton, M., Vieira, P., Hsieh, C. S., Culpepper, J. A., Wysocka, M., Trinchieri, G., Murphy, K. M., and O'Garra, A., *J. Immunol.* **154,** 5071, 1995.
8. Mengel, J., Dare, L., Dare, G. M., Delgado, M., Nomizo, A., Silva, J. S., and Campos, N. A., *Eur. J. Immunol.* **22,** 3173, 1992.
9. Kobayashi, M., Fitz, L., Ryan, M., Hewick, R. M., Clark, S. C., Chan, S., Loudon, R., Sherman, F., Perussia, B., and Trinchieri, G., *J. Exp. Med.* **170,** 827, 1989.
10. Chan, S. H., Perussia, B., Gupta, J. W., Kobayashi, M., Pospisil, M., Young, H. A., Wolf, S. F., Young, D., Clark, S. C., and Trinchieri, G., *J. Exp. Med.* **173,** 869, 1991.
11. Gazzinelli, R. T., Wysocka, M., Hayashi, S., Denkers, E. Y., Hieny, S., Caspar, P., Trinchieri, G., and Sher. A., *J. Immunol.* **153,** 2533, 1994.
12. Skeen, M. J., Miller, M. A., Shinnick, T. M., and Ziegler, H. K., *J. Immunol.* **156,** 1196, 1996.
13. Manetti, R., Annunziato, F., Tomasevic, L., Gianno, V., Parronchi, P., Romagnani, S., and Maggi, E., *Eur. J. Immunol.* **25,** 2656, 1995.
14. Klinman, D. M., Yi, A.-K., Beaucage, S. L., Conover, J., and Krieg, A. M., *Proc. Natl. Acad. Sci. USA* **93,** 2879, 1996.
15. Magram, J., Connonghton, S. E., Warrier, R. R., Carjaval, D. M., Wu, C. Y., Ferrante, J., Stewart, C., Sarmiento, U., Faherty, D. A., and Gately, M. K., *Immunity* **4,** 471, 1996.

16. Sornasse, T., Flamand, V., De Becker, G., Bazin, H., Tielemans, F., Thielemans, K., Urbain, J., Leo, O., and Moser, M., *J. Exp. Med.* **175,** 15, 1992.
17. Muraille, E., De Becker, G., Bakkus, M., Thielemans, K., Urbain, J., Moser, M., and Leo, O., *Int. Immunol.* **7,** 295, 1995.
18. Rothlein, R., Dustin, M. L., Marlin, S. D., and Springer, T. A., *J. Immunol.* **137,** 1270, 1986.
19. Muller, W., and Vandenabeele, P., *Eur. J. Immunol.* **17,** 579, 1987.
20. Wilkinson, V. L., Warrier, R. R., Truitt, T. P., Nunes, P., Gately, M. K., and Presky, D. H., *J. Immunol. Methods* **189,** 15, 1996.
21. Pajak, B., and Danguy, A., *J. Fish. Biol.* **43,** 709, 1993.
22. Noelle, R. J., Roy, M., Shepherd, D. M., Stamenkovic, I., Ledbetter, J. A., and Aruffo, R., *Proc. Natl. Acad. Sci. USA* **89,** 6550, 1992.
23. Kennedy, M. K., Picha, K. S., Fanslow, W. C., Grabstein, K. H., Alderson, M. R., Clifford, K. N., Chin, W. A., and Mohler, K. M., *Eur. J. Immunol.* **26,** 370, 1996.
24. Koch, F., Stanzl, U., Jennewein, P., Janke, K., Heufler, C., Kampgen, E., Romani, N., and Schuler, G., *J. Exp. Med.* **184,** 741, 1996.
25. Cella, M., Scheidegger, D., Palmer-Lehmann, K., Lane, P., Lanzavecchia, A., and Alber, G., *J. Exp. Med.* **184,** 747, 1996.
26. Guery, J. C., Ria, F., Galbiati, F., and Adorini, L., *Eur. J. Immunol.* **27,** 1632, 1997.
27. Ma, X., Aste-Amezaga, M., Gri, G., Gerosa, F., and Trinchieri, G., *Chem. Immunol.* **68,** 1, 1997.
28. Naruo, K., Ichimori, Y., Koyama, M., and Tsukamoto, K., *J. Interferon Res.* **4,** 235, 1984.
29. Ito, Y., Tsurudome, M., Yamada, A., and Hishiyama, M., *J. Immunol.* **132,** 2440, 1984.
30. Varki, A., *FASEB J.* **11,** 248, 1997.
31. Balzarini, J., Neyts, J., Schols, D., Hosoya, M., Van-Damme, E., Peumans, W., and De Clerk, E., *Antiviral Res.* **18,** 191, 1992.
32. Ravindranath, R. M., and Graves, M. C., *J. Virol.* **64,** 5430, 1990.
33. Steuer, M. K., Beuth, J., Hofstadter, F., Probster, L., Ko, H. L., Pulverer, G., and Strutz, J., *Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis.* **282,** 287, 1995.
34. Cundell, D. R., Weiser, J. N., Shen, J., Young, A., and Tuomanen, E. I., *Infect. Immunol.* **63,** 757, 1995.
35. Fearon, D. T., and Locksley, R. M., *Science* **272,** 50, 1996.
36. Medzhitov, R., and Janeway, C. A., *Curr. Opin. Immunol.* **9,** 4, 1997.
37. Murphy, K. M., Murphy, T. L., Szabo, S. J., Jacobson, N. G., Guler, M. L., Gorham, J. D., Gubler, U., and Nutley, N. J., *Chem. Immunol.* **68,** 54, 1997.