should apply to larger and more complex molecules, particularly to those which fall in a given class, like the amino acids. Here I show that such a relationship exists for the naturally-occurring amino acids, and that it is suprisingly linear when the molecular volumes are defined as those which each side chain occupies (on the average) in tight packing in protein crystals.

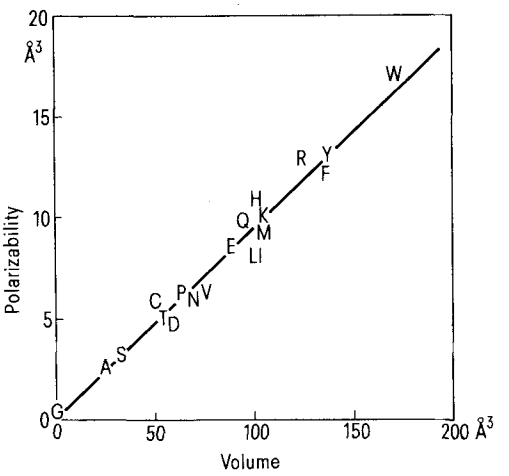
The molar refractions of the individual amino acid side chains were obtained by summing the appropriate group molar refractions, which were taken from the extensive tables of substituent coefficients for structure-activity correlations published by Hansch et al.[1]. The molar refractions were converted to polarizabilities by means of the Lorentz-Lorenz equation, with the usual assumption that the optical frequencies on which the molar refractions are based correspond to infinite frequency. The side chain volumes for the amino acids were calculated from the whole-residue packing volumes (the average volume occupied by a given residue in the interior of a protein)[2,3] by subtracting the packing volume of glycine from each of the published values. A plot of the calculated polarizabilities of the amino acids against their corresponding side chain packing volumes is shown in the figure. The data are best described by the straight line:

$$\alpha = 0.0726 + 0.0945\ V \simeq 0.0945\ V \qquad (1)$$

with a correlation coefficient of 0.96. The side chain volumes are 25 Å^3 or more, and neglecting the small first term leads to a maximum 3% discrepancy (alanine) as compared with the complete equation, so that within the limits of the treatment used, the polarizability of an amino acid side chain is directly proportional to its packing volume.

Among the possible areas in which the relation (1) might find application is in the ab initio calculation of 3-dimensional protein structures. The number of potential structures which must be evaluated in order to find the global energy minimum which corresponds to the most probable structure is enormous if the calculations are carried out on an atom-by-atom basis, and a considerable simplification of the problem is afforded by methods in which certain groups of atoms or whole side chains are treated as single entities[4]. The best sources of the van der Waals energy parameters for protein folding calculations appear to be those that use geometries based on single crystal data[5], so that semi-empirical relationships like the one discussed above may find some use in this application.

Similar linear relationships have been discovered between the hydrophobicity of the amino acid side chains and their crystal packing volumes[6], but here the data appear to fall into 2 classes, depending on whether a potential hydrogen-bonding heteroatom is present or not. Clearly van der Waals interactions (dispersion forces) between side chains are responsible for a significant fraction of the side chain contribution to so-called hydrophobic forces, and the linearity of 'hydrophobicity' with side chain packing volume is not surprising in the light of the linearity of the side chain polarizability with packing volume.



Polarizability of the amino acid side chains as a function of their side chain packing volumes. Amino acids included in the fitted curve are: Trp (W), Tyr (Y), Phe (F), Arg (R), Lys (K), Met (M), Ile (I), Leu (L), His (H), Gln (Q), Glu (E), Val (V), Asn (N), Pro (P), Asp (D), Thr (T), Cys (C), Ser (S), Ala (A), Gly (G).

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Mixed lymphocyte reaction in human monoclonal gammopathies[1]

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Summary. Ten multiple myeloma (MM) and 5 monoclonal gammopathies of undetermined significance (MGUS) were studied. The mixed lymphocyte reaction (MLR) proliferative response was of the same order of magnitude in MM and in MGUS as in normal controls. Normal results were obtained when pathological lymphocytes were used as either responding or stimulating cells. The addition of monoclonal anti-Ia antibodies completely abrogates the proliferative response as in healthy individuals. These results suggest that the immunological mechanisms supposed to be important in MLR are functionally normal in MM and in MGUS, although both the B and T cell lineages are involved in monoclonal gammopathies.

In multiple myeloma (MM) and in monoclonal gammopathy of undetermined significance (MGUS), a great number of lymphocytes displaying the same idiotypic specificities was demonstrated by using antisera directed against the idiotypic specificities of the M component[3-8]. A lymphocyte subset, rosetting with sheep red blood cells (SRBC), devoid

of immunoglobulin receptors but nevertheless displaying membrane idiotypic specificities has also been detected[9-11]. Moreover, in MM and in MGUS both the B and T cell lineages are involved in the disease.

In the present study, some characteristics of the peripheral blood lymphocytes were studied in the mixed lymphocyte reaction (MLR) system, by using pathological lymphocytes as either responding or stimulating cells. In addition, an attempt was made to inhibit this response by monoclonal anti-Ia antibodies.

The MLR can be defined as the T lymphocyte proliferation induced by interaction between allogeneic lymphocytes in vitro[12]. This response primarily involves T lymphocytes directed against polymorphic determinants coded by the HLA D/DR region[12,13]; these determinants represent the human equivalent of murine Ia antigen[14]. Thereafter, inhibition of allogeneic MLR by xenoantisera to Ia molecules[15] and more recently by monoclonal anti-Ia antibodies[16] has been reported, even if the mechanisms involved in this phenomenon are not completely clarified[16].

Ten IgGK multiple myeloma were studied before chemotherapy; diagnosis was made according to the proposed criteria[17]. Five asymptomatic patients had a stable monoclonal peak for more than 3 years and were classified as monoclonal gammopathies of undetermined significance[18].

Peripheral blood mononuclear cells (PBMC) of normal and pathological subjects were isolated by Ficoll-Hypaque density gradient centrifugation and depleted of monocytes by removal of cells adhering to plastic Petri dishes. 4–5 ml of PBMC suspension (1×10^6 cells/ml) in RPMI containing 10% heat-inactivated pooled human AB serum were incubated on plastic dishes for 60 min at 37 °C in a humidified atmosphere containing 5% CO_2. The nonadherent cells were harvested after swirling of the plates; the plates were then gently swirled with an additional 5 ml of warm RPMI to harvest the remaining nonadherent cells. The adherent layer was typically > 90% peroxidase positive. T cells were isolated from PBMC on Ficoll-Hypaque gradients according to their capacity to form rosettes with SRBC. Briefly, phagocyte-depleted PBMC (< 10% peroxidase positive) were spun down, adjusted to a concentration of 4×10^6 cells/ml and mixed with a 1% suspension of washed SRBC (1/1, v/v). E-rosetting T lymphocytes were separated from non-rosetting cells by Ficoll-Hypaque density gradient centrifugation: the interphase non-rosetting cells were named 'non-T' cells.

Unidirectional MLR were set up, in triplicate, in round bottomed microwell plates (Falcon, Becton Dickinson, CA), each culture consisting of 0.2 ml of RPMI 1640 medium (GIBCO) supplemented with 10% heat-inactivated pooled human AB serum, containing 1×10^5 responding T lymphocytes with an equal number of allogeneic stimulating non-T lymphocytes. The non-T cells were first rendered incapable of DNA synthesis by exposure to 3000 rad. Control samples included responding or stimulating cells cultured alone. Pathological lymphocytes were used both as responding cells against normal irradiated cells or as stimulating cells against normal responding cells. Cultures were

Table 1. Pathological T lymphocytes used as responding cells against normal irradiated non-T lymphocytes

Stimulating cells	Responding cells Pathological T cells	Antibody added None	PX63	D1-12	D4-22
Normal non-T cells	1 (MGUS)	65800±4900[a]	63800±3000	1200±370	1650±180
Normal non-T cells	2 (MGUS)	48600±5890	46800±2650	870±230	n.d.[b]
Normal non-T cells	3 (MGUS)	43650±4840	44590±4200	1950±310	2040±350
Normal non-T cells	4 (MGUS)	35220±2010	37870±2150	1050±135	1230±250
Normal non-T cells	5 (MGUS)	43840±2190	45835±3090	n.d.	2990±240
Normal non-T cells	1 (MM)	45000±3750	43550±4800	770±120	1150±350
Normal non-T cells	2 (MM)	37500±2400	38450±3420	380±80	650±125
Normal non-T cells	3 (MM)	39251±3090	42840±4570	1120±135	1402±247
Normal non-T cells	4 (MM)	22148±1990	n.d.	895±48	1302±104
Normal non-T cells	5 (MM)	26740±3265	28930±2730	2140±750	2555±890
Normal non-T cells	6 (MM)	37770±4010	39850±3750	1570±640	1900±290
Normal non-T cells	7 (MM)	45808±1640	44390±2850	2740±450	3400±900
Normal non-T cells	8 (MM)	48000±3890	46740±3570	1090±672	n.d.
Normal non-T cells	9 (MM)	43060±2140	42990±3800	2000±450	n.d.
Normal non-T cells	10 (MM)	32810±3050	34000±2890	1110±380	1440±105
Normal control		51275±4290[c]	42750±2790	2750±940	3100±470

[a] Values are expressed as mean cpm ± SD of triplicate culture; [b] not done; [c] mean values of 6 normal subjects.

Table 2. Pathological irradiated non-T lymphocytes used as stimulating cells against normal responding T lymphocytes

Stimulating cells Pathological non-T cells	Responding cells	Antibody added None	PX63	D1-12	D4-22
1 (MGUS)	Normal T cells	26800±1800[a]	27360±2400	1111±280	1950±330
2 (MGUS)	Normal T cells	39700±2780	38600±1500	1800±230	2100±290
3 (MGUS)	Normal T cells	32870±3074	n.d.[b]	2105±420	2380±160
4 (MGUS)	Normal T cells	31750±1950	32750±2690	1550±370	1900±254
5 (MGUS)	Normal T cells	48860±1900	48000±2050	n.d.	2320±300
1 (MM)	Normal T cells	45000±2780	43800±3645	2900±250	n.d.
2 (MM)	Normal T cells	26500±2500	n.d.	2500±470	2800±500
3 (MM)	Normal T cells	29780±3000	30100±2050	1740±111	1905±330
4 (MM)	Normal T cells	47080±3024	n.d.	1404±270	1550±211
5 (MM)	Normal T cells	29750±2090	30110±3400	n.d.	2745±104
6 (MM)	Normal T cells	35078±2500	34900±2490	1200±125	1480±198
Normal control		51275±4290[c]	42750±2790	2750±940	3100±470

[a] Values are expressed as mean cpm ± SD of triplicate culture; [b] not done; [c] mean values of 6 normal subjects.

incubated at 37 °C in a 5% CO_2 and 95% humidified air environment for 7 days. Proliferation was evaluated by adding 1 μCi/well of H^3TdR (sp. act. 50 mCi/ml) 18 h before harvesting.

Anti-Ia monoclonal antibodies were produced as previously reported[19]. Briefly, the hybridoma were obtained by fusing spleen cells from Balb/c mice, immunized with membrane-enriched fraction from the Daudi human lymphoblastoid cell line, with the mouse myeloma P3-X63Ag8 (D1-12 hybridoma) and P3-NS1Ag4 (D4-22 hybridoma). In studies of the effect of adding anti-Ia monoclonal antibodies to MLR, 50 μl of either D1-12 or D4-22 hybridoma culture fluid were added at the onset of the MLR.

The MLR proliferative response was of the same order of magnitude in MM and in MGUS as in normal controls. This result was the same both when pathological lymphocytes were used as responding cells (table 1) or as stimulating cells (table 2).

Moreover, the addition of either one of the monoclonal anti-Ia antibodies at the onset of the MLR resulted in more than 80% of inhibition of the proliferative response. In contrast, culture fluids from the parental myeloma PX63 had no inhibitory effect.

Although the biological significance of the MLR is not completely determined, it is evident that it reflects an important role played in vivo by the immune system[12, 13, 20]. An impaired MLR has been demonstrated by several workers in diseases characterized by immunodeficiencies, as Hodgkin's disease[21]. By analogy, in MM where the immunodeficiency is well-known, and B and T cell populations are involved in the disease[5-11], similar impairment would be expected. However, our data show proliferative values in the normal range when pathological cells are used as either responding or stimulating cells.

Moreover, when pathological lymphocytes were used as stimulating cells, a normal inhibition of the MLR by monoclonal anti-Ia antibodies was obtained. Since the MLR proliferative response can be abrogated by anti-Ia antibodies in MM and in MGUS as in normal controls, we suggest that the expression of Ia molecules on the surface of pathological lymphocytes is not altered and also the immunological mechanisms responsible for this inhibition are functionally normal.

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Antibodies from patients with liver diseases and from normal human or animal sera against glutaraldehyde-polymerized albumins: lack of species specificity

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Summary. By the use of glutaraldehyde-polymerized albumins of different species (human, rabbit, bovine, goat and mouse) it was demonstrated that anti-albumin antibodies in sera of patients with liver diseases and in normal human and animal (rabbit, mouse) sera are not species specific.

Antibodies against glutaraldehyde(GA)-polymerized human serum albumin have been described in patients with different liver diseases[2-6], and have been shown to be specific for the new antigenic determinants induced in albumin by GA treatment[7]. Anti-albumin antibodies (AAA) have also been found, in low concentrations, in normal human[8], mouse[9] and rabbit[10] sera. Unlike the receptor for polymerized albumin on hepatitis B surface antigen(HBsAg)-carrying particles, and the Clq component of complement, both of which bind polymerized human serum albumin in a species-restricted manner[11,12], AAA from pathological sera seem to react with a non-species-specific determinant on the polymerized human serum albumin molecule[13]. In this paper we report results which substantiate the latter supposition, bringing quantitative arguments that AAA from different species are not species specific.

Material and methods. Human serum albumin (HSA), rabbit serum albumin (RSA) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO,